

CHAPTER

# 11

## Urinalysis and Body Fluids

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## I. INTRODUCTION TO URINALYSIS

### A. Introduction

1. Urinalysis is the practice of examining urine for diagnostic purposes; it aids in following the course or treatment of disease.

### B. Importance of Urine

1. Urine **contains** most of the body's **waste products**.
2. Urine chemical changes are directly related to pathologic conditions.
3. A complete urinalysis is composed of multiple tests, including **physical, chemical, and microscopic** analysis.
4. Urinalysis is used for disease diagnosis, disease monitoring, drug screening, and initial diagnosis of inborn errors of metabolism.

### C. Urine Composition

1. Urine contains **mostly water** and various amounts of dissolved organic/inorganic compounds.
2. Composition varies according to diet, physical activity, metabolism, and disease processes. Composition is directly related to the amount and type of waste material that is to be excreted.
3. Urine **organic** substances
  - a. **Urea** accounts for roughly 50% of all dissolved solids in the urine.
  - b. Other organic substances in relatively large amounts include **creatinine** and **uric acid**.
  - c. Organic substances in small amounts include **glucose, protein, hormones, vitamins, and metabolized medications**.
4. Urine **inorganic** substances (listed in order of highest to lowest average concentration)
  - a. **Chloride, sodium, and potassium**
  - b. Other inorganic substances in small amounts include **sulfate, phosphate, ammonium, calcium, and magnesium**.
5. Nondissolved substances may include **bacteria, crystals, casts, mucus, and various types of cells**.

## II. THE KIDNEY AND URINE FORMATION

### A. Renal Anatomy

1. The **kidneys** are two bean-shaped organs located under the diaphragm on either side of the aorta in the posterior, upper abdominal region.
2. The **ureter** is a muscular tube that connects the pelvis of the kidney to the bladder.
3. Urine is stored in the **bladder** until excretion through the **urethra**.
4. The **renal pelvis** is a cavity area that is an expansion of the ureter. The pelvis functions to collect urine from the calyces for transport from the kidney to the ureter.

5. The kidneys consist of two regions, the **cortex** (outer layer) and the **medulla** (inner layer). The cortex is comprised of the renal corpuscles and the proximal and distal convoluted tubules of the nephron. The medulla is comprised of the loops of Henle and the collecting ducts.
6. The **abdominal aorta** supplies blood to the **renal artery**, which in turn provides blood to the kidney, and the **renal vein** functions to return blood to the **inferior vena cava**.
7. Microscopically, the functional unit of the kidney is the **nephron**, which is responsible for **urine formation**. It is comprised of a renal corpuscle and a tubular system. These areas are further delineated with the **renal corpuscle** consisting of the glomerulus and Bowman's capsule and the **tubular system** consisting of the proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct. More than a million nephrons may be found in each kidney.
  - a. The **glomerulus** is a tuft of capillaries that lie in a tubular depression called Bowman's capsule. The **afferent arteriole** carries blood into the glomerulus, and the **efferent arteriole** carries blood away. The **peritubular capillaries**, which arise from the efferent arteriole, aid in the tubular reabsorption process by surrounding the various segments of the renal tubule. The main function of the glomerulus is to filter the blood.
  - b. The **proximal convoluted tubule** is located in the cortex.
  - c. The **loop of Henle** begins in the cortex, with the descending limb of the loop extending into the medulla where the bend of the loop is formed that then becomes the ascending limb, which ends in the cortex.
  - d. The **distal convoluted tubule** (DCT) is located in the cortex, and DCTs from multiple nephrons direct the urine flow into a collecting duct.
  - e. The **collecting duct** joins with other collecting ducts, forming a papillary duct to carry urine into a calyx of the **renal pelvis**.

## B. Renal Physiology

1. In order to form and excrete urine, three processes function together: glomerular filtration, tubular reabsorption, and tubular secretion.
  - a. The glomerulus functions as a semipermeable membrane to make an **ultrafiltrate** of plasma that is protein free.
    - 1) Large molecules (proteins, cells) remain in the arterioles, whereas smaller molecules (glucose, urea, sodium, chloride, potassium, bicarbonate, calcium, etc.) pass through the glomerular capillary walls to become part of the filtrate.
    - 2) These smaller molecules and ions flow into the proximal convoluted tubule.
    - 3) The **glomerular filtration rate (GFR)** is about 115–125 mL of filtrate formed per minute by the glomeruli. The **renal tubules** will **reabsorb** all but 1 mL of the filtrate, which will be passed in the urine.

- b. **Reabsorption** is the process by which filtered water, ions, and molecules leave the tubules for return to the blood via the peritubular capillaries.
  - c. **Secretion** is the process by which a substance from the blood is transported across the wall of the tubule into the filtrate.
2. Function of the nephron
- a. **The proximal convoluted tubule**
    - 1) Responsible for most of the reabsorption (approximately 65%) and secretion that occurs in the tubules
    - 2) For some analytes, there is a limitation as to how much solute can be reabsorbed. This is defined as the “**renal threshold**.”
    - 3) **Reabsorbs** water,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ , urea, glucose (up to renal threshold of 160–180 mg/dL), amino acids, etc.
    - 4) **Secretes** hydrogen ions and medications
  - b. **Loop of Henle**
    - 1) **Descending limb** reabsorbs water.
    - 2) **Ascending limb** reabsorbs  $\text{Na}^+$  and  $\text{Cl}^-$ .
    - 3) **Filtrate leaves the loop of Henle** and moves into the distal convoluted tubule.
    - 4) Approximately 85% of tubular reabsorption of water and salt is completed before the filtrate passes into the distal convoluted tubule.
  - c. **Distal convoluted tubule**
    - 1) Reabsorbs  $\text{Na}^+$
    - 2) Reabsorption of water controlled by **antidiuretic hormone** (ADH)
    - 3) Secretion of  $\text{H}^+$  and  $\text{K}^+$
    - 4) **Aldosterone** controls the reabsorption of sodium and water and secretion of potassium and hydrogen into the filtrate.
  - d. **Collecting duct**
    - 1) Final site for water reabsorption to make urine more dilute or concentrated
    - 2)  $\text{Na}^+$  and  $\text{Cl}^-$  reabsorption controlled by **aldosterone**
    - 3) Water reabsorption occurs by osmosis as well as in response to **ADH**.

### C. Endocrine Functions

- 1. **Renin-angiotensin-aldosterone axis**
  - a. **Renin** is secreted by the juxtaglomerular apparatus of the **kidneys** and catalyzes the conversion of angiotensinogen to **angiotensin I** (a hormone in the inactive form).
  - b. Angiotensin I stimulates the production of **angiotensin II** (a hormone in the active form).
  - c. **Angiotensin II** regulates renal blood by:
    - 1) **Constriction** of renal arterioles
    - 2) **Secretion** of **aldosterone** from the adrenal glands to facilitate retention of sodium.

2. **Aldosterone**, made in the cortex of the adrenal glands, acts on the kidneys by promoting the reabsorption of  $\text{Na}^+$  from the filtrate into the blood and the secretion of  $\text{K}^+$  from the blood into the filtrate. Water will be reabsorbed along with the  $\text{Na}^+$ .
3. **Antidiuretic hormone (ADH)**, secreted by the posterior pituitary gland, promotes water reabsorption from the filtrate into the blood. ADH primarily affects the reabsorption of water from the distal convoluted tubule and the collecting duct.
4. **Parathyroid hormone (PTH)**, made in the parathyroid glands, promotes  $\text{Ca}^{2+}$  reabsorption from the filtrate into the blood and excretion of phosphate ions from the blood into the filtrate.
5. Erythropoietin is an alpha-globulin produced by the peritubular fibroblasts in the kidneys to stimulate red blood cell production in response to lowered oxygen levels.

### III. RENAL PATHOLOGY AND RENAL FUNCTION TESTS

#### A. Renal Pathology

1. **Acute glomerulonephritis**: Inflammation of the glomerulus seen in children and young adults; can follow a Group A Streptococcus respiratory infection; characterized by **hematuria, proteinuria, WBCs, and casts** (RBC, granular and hyaline)
2. **Rapidly progressive glomerulonephritis**: A more serious condition than acute glomerulonephritis that may result in renal failure; urinalysis results would be similar to acute glomerulonephritis
3. **Acute interstitial nephritis**: Inflammation of the renal interstitium that may be caused by an allergic reaction to medication; characterized by **hematuria, proteinuria, WBCs** (especially eosinophils), and **WBC casts**
4. **Membranous glomerulonephritis**: Thickening of the glomerular capillary walls and basement membrane; characterized by **hematuria and proteinuria**
5. **Nephrotic syndrome**: May be caused by renal blood pressure irregularities; characterized by **proteinuria ( $>3.5 \text{ g/24 hr}$ ), hematuria, lipiduria, oval fat bodies, renal tubular epithelial cells, and epithelial, fatty, and waxy casts**
6. **Focal segmental glomerulosclerosis**: Affects a specific number of glomeruli, not the entire glomerulus; often seen in HIV patients; characterized by **hematuria and proteinuria**
7. **Chronic glomerulonephritis (Berger disease)**: Results in a long-term progressive loss of renal function; characterized by **hematuria, proteinuria, glucosuria, presence of casts, including broad casts**
8. **Acute pyelonephritis**: An infection of the renal tubules caused by a urinary tract infection; characterized by **hematuria, proteinuria, WBC's, bacteria, and WBC and bacterial casts**

9. **Chronic pyelonephritis:** Chronic infection of the tubules and interstitial tissue that may progress to renal failure; characterized by **hematuria, proteinuria, WBCs, bacteria, and WBC, bacterial, granular, waxy, and broad casts**
10. **Renal failure:** Tubular necrosis caused by nephrotoxic agents and other disease processes, resulting in a **failure of the kidneys to filter blood**

## B. Renal Function Tests

1. **Renal tubular reabsorption tests** (also known as **concentration tests**) are used to detect early renal disease. Examples of these tests include:
    - a. **Osmolality** measures the **amount of solute dissolved** in a solution.
    - b. **Specific gravity** depends on the **solute dissolved** in a solution **and the density** of this solute. **Osmolality and specific gravity** evaluate renal concentrating ability, monitor the course of renal disease, and monitor fluid and electrolyte therapy.
    - c. **Osmolar/Free Water Clearance**
      - 1) Used in the diagnosis of various types of diabetes mellitus
      - 2) Measures renal clearance of solutes and substance-free water
  2. **Secretion test**
    - a. **Tubular secretion or renal blood flow test** uses ***p*-aminohippuric acid (PAH)**, a substance that is infused into the patient.
    - b. PAH is completely removed from the blood by functional renal tissue. If renal problems exist, the PAH will not be removed completely.
  3. **Glomerular tests** are used to assess renal **waste removal** and solute **reabsorbing abilities**. A decreased clearance test indicates compromised kidney function.
- Note:** Urea is not normally used in clearance testing because of tubular reabsorption, diet, and urine flow rate.
- a. **Creatinine clearance** is used to **assess glomerular filtration rate**.
    - 1) Creatinine levels are not changed by diet (normal) or rate of urine flow. Creatinine is not reabsorbed by renal tubules. **P:** plasma creatinine mg/dL, **U:** urine creatinine mg/dL, **V:** urine flow in mL/min, and **SA:** body surface area. 1.73 m<sup>2</sup> is average body surface area.
    - 2) **Creatinine clearance formula:**

$$C \text{ (mL/min)} = \frac{U \times V}{P} \times \frac{1.73 \text{ m}^2}{SA}$$

- 3) **24-hour timed urine** is the specimen of choice.
- 4) **Reference ranges** differ according to age and sex; values decrease with age.

Males: 105 ± 20 mL/min/1.73 m<sup>2</sup>

Females: 95 ± 20 mL/min/1.73 m<sup>2</sup>

- b. **Estimated glomerular filtration rate (eGFR)** uses only a **blood creatinine** and the **MDRD** (modification of diet in renal disease) **formula**.
  - 1) Correction for gender and race required
  - 2) Results only reported as a number if  $<60 \text{ mL/min/1.73 m}^2$

#### IV. URINE VOLUME AND SAMPLE HANDLING

##### A. Volume of Urine

1. Determined by the body's state of hydration; normal range of urine output 600–2000 mL/24 hr, with **1200–1500 mL/24 hr** being the **normal adult output**
2. **Factors that affect urine volume**
  - a. Fluid intake and fluid loss related to **nonrenal** functions
  - b. Diuretic and antidiuretic **hormone** levels
  - c. Excretion of dissolved solids, including **glucose** and **salts**
3. Urine volume terminology
  - a. **Oliguria: Decrease in urine output** because of dehydration (e.g., vomiting, diarrhea, perspiration, and burns)
  - b. **Anuria: No urine output** because of kidney damage or renal failure
  - c. **Nocturia: Increased urine output at night**
    - 1) Caused by a reduction in bladder capacity resulting from pregnancy, stones, or prostate enlargement
    - 2) Increased fluid intake at night
  - d. **Polyuria: Increased daily output**, may exceed 2 L/day
    - 1) Usually **caused by** such diseases as **diabetes mellitus** and **diabetes insipidus**
    - 2) May also be caused by **ingestion of diuretics** (medications that block water reabsorption), **caffeine**, and **alcohol** (suppresses secretion of antidiuretic hormone)

##### B. Specimen Collection and Handling

1. Urine must be treated as biohazardous material, thus warranting that **standard precautions** be followed.
2. Use clean, dry cups with lids.
3. Label container with name, identification number, and the date and time of urine collection.
4. Analyze within **2 hours** or preserve.
5. **Preserve** urine
  - a. Refrigeration will **decrease** bacterial growth but will cause precipitation of amorphous phosphates/urates.
  - b. **Before testing**, urine must be brought to **room temperature**.
  - c. **Chemical preservatives** can be bactericidal, but they will preserve formed elements and will not, generally, interfere with chemical testing. If the chemical preservative alters the pH, it can interfere with the pH test on the strips.



- d. Changes in unpreserved urine include:
  - 1) **Increased** pH, nitrites, bacteria, and turbidity
  - 2) **Decreased** urobilinogen, glucose, ketones, and bilirubin
  - 3) Formed element destruction
  - 4) Change in color
- 6. Urine specimen types and collection times
  - a. **First morning:** Concentrated specimen used for routine screening, pregnancy tests, and for detecting orthostatic proteinuria
  - b. **Random midstream clean-catch:** Used for routine screening and microalbuminuria determination; time of collection not a consideration; patient cleans the external urinary meatus, urinates a small volume into the toilet, and then collects the rest of the voided sample.
  - c. **Fasting:** Used for glucose monitoring; usually the second voided specimen of the morning following a fasting period
  - d. **2-Hour postprandial:** Void 2 hours after eating; used to monitor glucose content
  - e. **24-Hour:** Collected over a period of 24 hours for **creatinine clearance** or for quantifying other analytes, including  $\text{Na}^+$  and  $\text{K}^+$
  - f. **Catheterized:** Collected from a tube placed through the urethra into the bladder; used for bacterial culture and routine screening
  - g. **Midstream clean-catch:** Genital area cleansed with a detergent and urine collected in the middle of urination; used for bacterial culture and is the preferred collection method for routine urinalysis because it is the most concentrated sample of the day.
  - h. **Suprapubic aspiration:** Needle inserted into the bladder through the abdominal wall; used for bacterial culture and cytologic testing
  - i. **Pediatric collection:** Use small, clear plastic bags with adhesive to adhere to the genital area

## V. PHYSICAL EXAMINATION OF URINE

### A. Color, Appearance, and Odor

- 1. **Urine color** varies from colorless to any color shade (black, red, green, etc.). Changes in color can be due to normal metabolism, disease, diet, and physical activity.
  - a. The normal color of urine (yellow) is derived from **urochrome**, which is a pigmented substance excreted at a constant rate. Increased urochrome production can result from thyroid disease or a fasting urine sample.
  - b. **Urobilin**, formed from the oxidation of urobilinogen as urine stands, adds minimally to the normal yellow color. As urine sits unpreserved, the color deepens to orange-brown.



- c. **Uroerythrin** adds a **slight pink** pigment, mostly apparent following refrigeration, when the pigment attaches to precipitated amorphous urates.
  - d. **Pale yellow** (straw) samples are generally **dilute**, whereas **dark yellow** samples are usually **concentrated**.
2. **Abnormal urine color**
- a. **Colorless/pale yellow**
    - 1) Random specimen
    - 2) Diabetes insipidus or diabetes mellitus with increased urine excretion
  - b. **Dark yellow**
    - 1) Concentrated specimen: First morning or following strenuous exercise
    - 2) Caused by dehydration from fever, burns, etc.
    - 3) Concentrated urine will also exhibit high specific gravity.
  - c. **Intense yellow/amber/orange**
    - 1) **Pyridium** (phenazopyridine): This medication is prescribed for **urinary tract infections** (UTIs); resulting **thick orange urine** will mask chemical and microscopic analysis.
    - 2) **Bilirubin: Bilirubinemia** occurs from liver problems, such as hepatitis, biliary obstruction, etc. With an increase of bilirubin in the blood, bilirubinuria occurs; **yellow foam** forms when **urine** is **shaken** due to the presence of **conjugated bilirubin**.
  - d. **Red/pink**
    - 1) Blood: Glomerular bleeding can also produce brown/black urine.
    - 2) Hemoglobin, erythrocytes
    - 3) Myoglobin (muscle trauma)
    - 4) Porphyrins
  - e. **Green/blue**
    - 1) Medications and dyes such as amitriptyline, indican, and phenols
    - 2) Infections caused by *Pseudomonas* species
  - f. **Brown/black**
    - 1) Denatured hemoglobin, melanin, or homogentisic acid
- Note:** Many abnormal colors are nonpathogenic in nature and are the result of food, drugs, or vitamins.
3. **Appearance of urine** (clarity): The visual inspection of urine uses the following terminology: clear, hazy, slightly cloudy, cloudy, turbid, milky, and bloody.
- a. **Clear:** Indicates the absence of significant numbers of formed elements
  - b. **Hazy/slightly cloudy:** May be due to the presence of low numbers of formed elements

- c. **Cloudy:** In acid urine, may be due to amorphous urates showing a slight pink color, calcium oxalate crystals, or uric acid crystals; in alkaline urine, if the sample is white, may be due to amorphous phosphates and carbonates
- d. **Turbid:** May be due to the presence of large numbers of formed elements
- 4. **Urine odor** is not generally a part of the routine urinalysis but may provide useful information to the physician.
  - a. Aromatic odor: Normal
  - b. Ammonia odor: Urea metabolized by bacteria into ammonia
  - c. Strong odor: Bacterial infection
  - d. Sweet or fruity odor: Ketone bodies (diabetic ketosis)
  - e. Maple syrup odor: Maple syrup urine disease
  - f. Various odors: Different foods

## B. Specific Gravity

- 1. Specific gravity determines the **kidney's ability to reabsorb** essential chemicals and water from the glomerular filtrate. **Reabsorption** is the first renal function to become impaired. Specific gravity also detects dehydration and antidiuretic hormone abnormalities.
- 2. Specific gravity is the density of a substance compared with the density of a similar volume of deionized water at a similar temperature and is influenced by the number of particles dissolved and by particle size.
- 3. **Specific gravity instrument: Refractometer** measures a refractive index, by comparing the **velocity of light in air to the velocity of light in a solution**. Method uses a small volume of urine and does not require temperature corrections.
- 4. **Specific gravity (S.G.) values: Plasma filtrate** entering the glomerulus has a **S.G. of 1.010**.
  - a. **Normal** random urine **ranges** from **1.003 to 1.035**, with the average S.G. falling between 1.015 and 1.025.
  - b. **Isosthenuric** urine: **1.010** (fixed S.G. indicates loss of concentrating and diluting ability)
  - c. **Hyposthenuric** urine: **Less than 1.010**
  - d. **Hypersthenuric** urine: **Greater than 1.010**
- 5. Conditions associated with specific gravity value
  - a. **Low specific gravity** indicates **loss** of the kidney's **ability to concentrate** urine or presence of **disease**, such as diabetes insipidus, glomerulonephritis, and pyelonephritis. It can also be found normally if the person has a large fluid intake.
  - b. **High specific gravity** may result from adrenal insufficiency, diabetes mellitus, hepatic disease, congestive heart failure, and dehydration due to vomiting, diarrhea, low fluid intake, or strenuous exercise.
  - c. **Interference from X-ray contrast media** excretion may result in **S.G. > 1.035**.

## VI. CHEMICAL EXAMINATION OF URINE

### A. Reagent Strips

1. Reagent strips are used for the following **tests**: pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, leukocytes, creatinine, and specific gravity. Reagent strips are the method of choice for the chemical analysis of urine.
2. **Basic use**: Reagent strips are **chemical-containing absorbent pads** that react with urine, producing a chemical reaction that results in a color change. The color is characteristic of positive reactions for various substances. Color intensity is **semiquantitative** for these substances. Confirmatory tests are then performed for some analytes.
3. **Sources of error** in reagent strip use include excess time in the urine, runover between chemicals, not following specific reaction times to read results, and not testing samples at room temperature.
4. Reagent strips should be assessed for accuracy by the use of “normal” and “abnormal” urine controls, run on at least a daily basis.

### B. Chemical Tests and Clinical Significance

1. **pH**
  - a. The **acid-base balance** of the body is primarily **regulated** by the **lungs** and the **kidneys**. The kidneys provide regulation through **secretion of hydrogen ions** via ammonium ions, hydrogen phosphate, and organic weak acids. The kidneys also facilitate the **reabsorption of bicarbonate** from the convoluted tubules.
  - b. The **pH** of urine ranges from **4.5 to 8.0** for random urines and from **5.0 to 6.0** for the first morning void. The following urine pHs may indicate various diseases and conditions:
    - 1) **Acidic pH (<6.0)**: High-protein diets, after normal sleep, respiratory/metabolic acidosis, uncontrolled diabetes mellitus
    - 2) **Alkaline pH (>7.0)**: Excreted after meals in response to gastric HCl, vomiting, increased consumption of vegetables, renal tubular acidosis, respiratory/metabolic alkalosis, and UTIs. Note that urine may also be alkaline as a result of delay in testing due to the action of bacteria on urea to form ammonia.
  - c. For **pH**, the **reagent strip** uses **methyl red and bromthymol blue** to detect changes in pH. At pH 5.0 the pad is orange; as the pH increases, the pad will go from orange to yellow to green, and finally to blue at pH 9.0.
2. **Protein**
  - a. The reagent strip uses **tetrabromphenol blue** to detect protein.
    - 1) Principle (“protein error of indicators”): The indicator is yellow in the absence of protein and the pad changes from **shades of green to blue** when **abnormal** amounts of protein (albumin) are present.

## 2) Reaction interference

- a) **False positives:** Urine pH >9, resulting from alkaline medicine and keeping the reagent strip in urine too long
- b) **False negatives:** Dilute urine; proteins other than albumin present
- b. Normal urine will contain **less than 10 mg/dL** of protein or **100 mg/24 hr**. Protein types include **albumin, microglobulins, and uromodulin** (Tamm-Horsfall).
- c. Urine protein is very diagnostic for renal disease and many indicate tubular reabsorption problems, increased low-molecular-weight proteins, and glomerular membrane damage caused by toxic agents, lupus, or *streptococcal glomerulonephritis*.
- d. Protein can come from **nonrenal sources**, such as prostate, seminal, and vaginal secretions. However, these other sources of protein will not usually be detectable by the reagent strip method, which is selective for albumin.
- e. **Bence Jones protein** is produced due to a **proliferative disorder of plasma cells** as seen in **multiple myeloma**. Bence Jones proteins are **light chain monoclonal immunoglobulins**.
- f. **Benign proteinuria** can occur in cold temperatures and as a result of exercise, fever, dehydration, late pregnancy, and orthostatic/postural proteinuria in young adults (going from supine to upright).
- g. **Microalbumin** evaluation is useful for patients with **renal complications of diabetes mellitus**. The protein levels found may be less than that detectable by routine protein reagent strip tests. Performed on random urines, microalbumin analysis always requires the simultaneous analysis of creatinine, and it is reported as an Alb/Cr ratio. **Abnormal** values (microalbuminuria) will be **30–299 mg albumin/g creatinine**.

## 3. Creatinine

- a. **Creatinine** testing in a random urine is performed only for **comparison with the protein level to rule out microalbuminuria**.
- b. Creatinine provides an assurance that the **water volume** of the sample is **not** influencing the protein concentration.
- c. **Methodology of creatinine testing**
  - 1) **Creatinine** in the urine sample forms a **complex with copper reagent** in the reagent strip pad. This complex has **pseudoperoxidase** activity that **catalyzes the oxidation of a chromagen** to a colored end product.
  - 2) The final concentration from the **creatinine** pad is **matched in a grid** with the concentration from the **protein** pad. The intersection of the two test results will indicate if the protein result is normal or abnormal.
  - 3) Reagent strips with the creatinine pad will commonly lack a urobilinogen pad.
  - 4) **Sensitivity** of the **creatinine** method is **10 mg/dL**, which will be **interpreted** as a specimen that is **too dilute to use for analysis**.
    - a) **False positives:** None
    - b) **False negatives:** Ascorbic acid

#### 4. Glucose

- a. **Glucose** testing is most commonly used to detect and monitor **diabetes mellitus**.
- b. **Glucosuria** is the presence of **urine glucose** and is seen in the following conditions: diabetes mellitus, impaired tubular reabsorption seen in Fanconi syndrome, advanced tubular renal disease, central nervous system (CNS) damage, thyroid disorders, and pregnancy.
- c. **Methodology of glucose testing**
  - 1) **Reagent strip:** Glucose + oxygen are catalyzed by **glucose oxidase** to form gluconic acid and  $\text{H}_2\text{O}_2$ . The  $\text{H}_2\text{O}_2$  + chromogen are catalyzed by **peroxidase** to form an oxidized colored chromogen + water.
    - a) **False positives:** Strong oxidizing agents (e.g., bleach)
    - b) **False negatives:** Ascorbic acid
  - 2) **Copper reduction test** (Benedict's, Clinitest tablet): Method utilizes a reduction reaction, in which **glucose** (or other reducing substances) **reduces copper sulfate** (blue) **to cuprous oxide** (various shades of yellow to green). Currently, this test is not used for confirming the presence of glucose, because it is much less sensitive to glucose than the strip test. Instead, this test is mainly used to detect the presence of galactose in urine for patients with galactosemia.
    - a) **False positives:** Antibiotics, ascorbic acid and other reducing sugars
    - b) **False negatives:** None

#### 5. Ketones

- a. Include three intermediate **products of fatty acid metabolism: acetone, acetoacetic acid, and beta-hydroxybutyric acid**
- b. **Normal** urine contains **no ketones** when metabolized fat is broken down completely, but when fat reserves are needed for energy, ketones will show up in the urine.
- c. **Ketonuria:** The presence of ketones in the urine
- d. **Clinical significance:** Uncontrolled diabetes mellitus, insulin dosage monitoring, electrolyte imbalance, and dehydration due to excessive carbohydrate loss such as vomiting, starvation, exercise, and rapid weight loss
- e. **Methodology of ketone testing**
  - 1) Reagent strips use **sodium nitroprusside** (nitroferricyanide) to measure **acetoacetic acid**. The addition of **glycine** permits the measurement of **acetone and acetoacetic acid**.
    - a) **False positives:** Pigmented urine, dyes, phenylketones
    - b) **False negatives:** Bacterial breakdown of acetoacetic acid
  - 2) **Acetest** is a **nitroprusside** and **glycine** tablet used to detect ketones; gives an enhanced color reaction and permits serial dilutions to be done. Reaction interference parallels the reagent strip method.
  - 3) The **enzymatic method** uses beta-hydroxybutyrate dehydrogenase to detect the presence of beta-hydroxybutyric acid.

## 6. Blood

- a. Reagent strip test detects **hematuria** and **hemoglobinuria**.
- b. Types of blood/hemoglobin in the urine
  - 1) **Hematuria (intact RBCs in the urine)**
    - a) Caused by renal calculi, glomerulonephritis, pyelonephritis, tumors, trauma, toxins, exercise, menstruation, and pregnancy
  - 2) **Hemoglobinuria (hemoglobin in the urine)**
    - a) Caused by transfusion reactions, hemolytic anemia, severe burns, infections, and exercise
  - 3) **Myoglobin** (hemoglobin-like protein found in **muscle tissue**)
    - a) The presence of **myoglobin** will cause a **positive reaction** on the reagent strip pad **for blood**. Myoglobin can be detected in muscle trauma, coma, convulsions, muscle-wasting diseases, and extensive exercise.
    - b) To **screen for myoglobin**, use **ammonium sulfate** to **precipitate hemoglobin** out of the urine; the **urine supernatant** is then filtered and **tested** with a reagent strip. **Positive reaction: myoglobin**; negative reaction: hemoglobin
- c. **Reagent strip methodology**
  - 1) Detects the **pseudoperoxidase** activity of **hemoglobin or myoglobin**;  $\text{H}_2\text{O}_2$  plus chromogen reacts with hemoglobin peroxidase to form oxidized chromogen and water. The chromogen is **tetramethylbenzidine**, which forms a green-blue color when oxidized.
  - 2) A **speckled pattern** on the reagent strip pad occurs when low numbers of **intact RBCs** lyse upon touching the reagent strip pad, releasing hemoglobin. **Free hemoglobin** or high numbers of red blood cells in the urine will form a **uniform color** on the pad.
- d. **Reaction interferences**
  - 1) **False positives:** Vegetable peroxidase and *Escherichia coli* peroxidase
  - 2) **False negatives:** High levels of ascorbic acid and nitrites, formalin preservatives, captopril (hypertension medication)

## 7. Bilirubin

- a. Detects **bilirubinuria**, a degradation product of hemoglobin
- b. Bilirubin is a pigmented yellow compound.
- c. Hemoglobin is metabolized into iron, protein, and **protoporphyrin**. The protoporphyrin is **converted to bilirubin** by reticuloendothelial system cells; bilirubin binds to albumin for transport to the liver as unconjugated bilirubin.
- d. In the liver, **bilirubin** is **conjugated** with **glucuronic acid** to form **bilirubin diglucuronide**, which goes to the intestines and is reduced to **urobilinogen** via bacterial action and excreted in the feces as **urobilin**. Urobilin gives feces their brown color. A small amount of urobilinogen reaches the kidney via the bloodstream and is excreted in the urine.



- e. **Bilirubinuria** may result from hepatitis, cirrhosis, biliary obstruction, and early liver disease when conjugated bilirubin enters the circulation.
    - 1) **Conjugated bilirubin** is **water soluble** and excreted in urine.
    - 2) **Unconjugated bilirubin** is *not* water soluble and cannot be excreted in urine.
  - f. **Bile duct obstruction** is positive for urine bilirubin (conjugated form increased in plasma) but normal for urine urobilinogen.
  - g. In **hemolytic disease**, urine urobilinogen is positive and urine bilirubin (unconjugated form increased in plasma) is negative.
  - h. **Reagent strip** uses **diazonium salt** reaction (bilirubin  $\rightarrow$  azobilirubin) methodology. The **Ictotest**<sup>®</sup> tablet is a diazo confirmatory test for bilirubin that is more sensitive and less subject to interference.
  - i. **Reaction interferences**
    - 1) **False positives:** Pigmented urine (i.e., medications, indican, and Iodine)
    - 2) **False negatives:** Specimen too old, excessive exposure to light (bilirubin exposed to light is converted to biliverdin, which does not react with diazonium salts), ascorbic acid, and nitrite
8. **Urobilinogen**
- a. Formed from hemoglobin metabolism; produced from the reduction of bilirubin by bacteria in the small intestine
  - b. Increased urobilinogen in the urine can indicate early liver disease, hepatitis, and hemolytic diseases.
  - c. Reagent strip uses **Ehrlich's reagent** (paradimethylaminobenzaldehyde) or a **diazo dye** to detect urobilinogen. Note that it is not possible to detect the absence of urobilinogen by the reagent strip.
  - d. **Reaction interferences**
    - 1) **False positives:** Pigmented urine
    - 2) **False decrease:** Improper storage, high levels of nitrite
9. **Nitrite**
- a. Rapid test for **UTIs**
  - b. A **positive nitrite** can indicate cystitis (bladder infection) and pyelonephritis.
  - c. Used for evaluation of UTI antibiotic therapy
  - d. **Reagent strip** detects the ability of **certain bacteria** to **reduce nitrate** (found in urine normally) **to nitrite** (abnormal in urine). In the reagent strip method, nitrite reacts with an aromatic amine to form a diazonium salt, which then reacts with a dye to produce a pink product.
  - e. **Reaction interferences**
    - 1) **False positives:** Old urine samples containing bacteria and pigmented urine
    - 2) **False negatives:** Ascorbic acid, antibiotics, bacteria that do not reduce nitrate, diet low in nitrates, inadequate time in bladder for reduction of nitrate to nitrite, and heavy concentration of bacteria that reduces nitrate all the way to nitrogen, which does not react



### 10. Leukocytes

- a. Indicate possible **urinary tract infection, inflammation** of urinary tract
- b. Reagent strip method does not quantify the number of WBCs.
- c. The strip method **detects lysed leukocytes** that would not be found under the microscope.
- d. **Reagent strip reaction:** An acid ester reacts with **leukocyte esterase** to form an aromatic compound that reacts with a diazonium salt, forming a purple color.
- e. **Reaction interferences**
  - 1) **False positives:** Pigmented urine, strong oxidizing agents
  - 2) **False negatives:** Increased glucose, protein, and ascorbic acid; yellow-pigmented substances; high specific gravity (prevents release of leukocyte esterases); lymphocytes (do not contain leukocyte esterase); selected antibiotics like gentamicin and tetracycline

### 11. Specific gravity

- a. Gives an approximate specific gravity value in increments of 0.005
- b. **Clinical significance:** Monitors hydration and dehydration, loss of renal tubular concentrating ability, and diabetes insipidus
- c. **Reagent strip reaction:** The **ionization of a polyelectrolyte** in an alkaline solution (due to change in dissociation constant) **produces hydrogen ions** proportionally to the ions present in the solution. This causes a **change in pH** that is detected as a decrease in pH, thus causing a **color change of bromthymol blue**, which is an indicator able to measure pH changes. S.G. = 1.000, pad color is blue in alkaline solution; S.G. = 1.030, pad color is yellow in acid solution. Note that the pad is sensitive only to ions in the urine. Cells and nonionized solutes like glucose will not cause a reaction.
- d. **Reaction interferences**
  - 1) **False positives:** Elevated protein or ketone levels increase the specific gravity.
  - 2) **False negatives:** If urine pH >6.5, add 0.005 to the reading.

## VII. MICROSCOPIC EXAMINATION OF URINE

### A. Microscopic Examination of Urine

1. Must be done to identify insoluble substances from the blood, kidney, lower urogenital tract, and external contaminants
2. **Formed elements:** Erythrocytes, leukocytes, epithelial cells, bacteria, yeast, fungal elements, parasites, mucus, sperm, crystals, and artifacts
3. Standard rules for microscopics
  - a. Examine urine while **fresh** or when **properly preserved**.
  - b. 10–12 mL of urine are centrifuged leaving 0.5–1.0 mL of sediment for viewing.

- c. Report RBCs/WBCs using high-power magnification (i.e., high-power field [hpf]); report casts and crystals using low-power magnification (i.e., low-power field [lpf]).
- d. All formed elements must be identified and quantified.
- e. Microscopic methods include brightfield, phase contrast, and polarized.

**B. Normal Urines:** Contain 0–2 RBCs (hpf), 0–5 WBCs (hpf), 0–2 hyaline casts (lpf), several epithelial cells (hpf), some types of crystals, and mucus. Note that the reference ranges for these will vary by the method and volume used.

### C. Urine Formed Elements

#### 1. Erythrocytes (7 microns)

- a. Number of cells counted is related to extent of renal damage, glomerular membrane damage, or urogenital tract vascular damage.
- b. RBCs associated with infections, toxins, cancer, circulatory problems, renal calculi, menstrual contamination, trauma, and exercise.
- c. **RBCs in normal urine appear as colorless disks;** in concentrated urine they **shrink** and appear **crenated**; in dilute or alkaline urine, RBCs **swell** and **lyse** with release of hemoglobin, leaving an **empty cell**, which appears as a **ghost cell**.
- d. RBCs can be confused with yeast cells or oil droplets (highly refractile). Dilute **acetic acid** can be used to **lyse RBCs**, leaving only yeast, oil droplets, and WBCs.

#### 2. Leukocytes (10–15 microns)

- a. **Pyuria** is **increased WBCs** in the urine and may indicate **infection** in the urogenital tract. Leukocytes in the urine may indicate the following:
  - 1) **Bacterial** infections, pyelonephritis, cystitis, prostatitis, and urethritis
  - 2) **Nonbacterial** pyuria resulting from glomerulonephritis, lupus, and tumors
- b. **Neutrophils** are the **predominant WBC** appearing in the urine with cytoplasmic granules and multilobed nuclei.
  - 1) **WBCs swell** in dilute alkaline urine (hypotonic), producing **glitter cells**, which have a sparkling appearance due to the Brownian movement of the granules.
  - 2) **Eosinophils** in the urine may indicate a drug-induced nephritis or renal transplant rejection. If suspected to be present, special staining will be needed to visualize these cells.

#### 3. Epithelial cells

- a. **Squamous epithelial (30–50 microns):** These cells are very common in the urine and usually not clinically significant. Squamous epithelial cells **line the lower urethra and vagina in women and the urethra of males**. They are the **largest** of the cells found in sediment with **abundant, irregular cytoplasm** and a **central nucleus** the size of a RBC. Excessive numbers of these cells may suggest the sample has not been collected

properly by the clean-catch method. In the presence of a vaginal infection, **clue cells** may appear; clue cells are squamous epithelial cells covered with *Gardnerella vaginalis* (coccobacillus).

- b. **Transitional epithelial:** These cells **line the renal pelvis, ureters, bladder, and upper urethra in males**. **Smaller** than squamous epithelial cells, transitional cells are **spherical/polyhedral/caudate** and have a **central nucleus**. There is no associated pathology, except in large numbers, with abnormal morphology, including vacuoles and irregular nuclei, which may indicate renal carcinoma or viral infection. Increased number of cells may be present after catheterization due to the invasiveness of the procedure.
  - c. **Renal tubular epithelial (RTE):** The RTE cell is the **most significant** epithelial cell in the urine. It has a **small eccentric nucleus**. Cell **size and shape vary** from rectangular, larger cells in the proximal convoluted tubule (PCT) to cells slightly larger than a WBC-shaped cuboidal or columnar originating from the collecting duct (CD). **Cytoplasm varies**, with the PCT being coarsely granulated and the CD being very finely granulated. **Tubular injury** is suggested when **> 5/hpf are present**. Can indicate renal cancer, renal tubular damage, pyelonephritis, toxic and allergic reactions, and viral infection. Types of renal tubular cells include:
    - 1) **Bubble cells** are RTE cells that contain large, non-lipid-filled vacuoles. These cells can be seen in **renal tubular necrosis**. Their presence is associated with dilation of endoplasmic reticulum before the death of injured cells.
    - 2) **Oval fat bodies** are renal tubular epithelial cells that have absorbed lipids that are highly refractile and stain with Sudan III or oil red O. They may indicate nephrotic syndrome.
  - d. **Miscellaneous cells: Histiocytes** in the urine may indicate lipid-storage disease. These cells are filled with fat and are larger than oval fat bodies.
4. **Casts**
- a. Of all the formed elements in the urine, only casts are **unique** to the kidney.
    - 1) Different casts represent different clinical conditions.
    - 2) **Cylindruria** is the term for casts in the urine.
    - 3) Casts are **formed** within the **lumen of the distal convoluted tubule** and **collecting duct**, taking on a shape similar to the tubular lumen. Their formation is favored when there is urinary stasis.
    - 4) Casts may have **formed elements** (such as bacteria, WBCs, RBCs, etc.) contained **within** them **or attached** to their surface.
    - 5) **Uromodulin** (Tamm-Horsfall glycoprotein) is the **major constituent of casts** and is poorly detected by reagent strip methods. Uromodulin is made by the **renal tubular epithelial cells** that line the DCT and upper CD. Casts also consist of some albumin and immunoglobulins.

## 5. Types of casts

- a. **Hyaline cast: Most commonly seen** cast, 0–5/lpf normal
  - 1) Increased hyaline casts normally follow exercise, dehydration, heat, and emotional stress.
  - 2) **Disease association:** Acute glomerulonephritis, pyelonephritis, chronic renal disease, and congestive heart failure
  - 3) **Appearance:** Colorless, with varied morphology
- b. **RBC cast:** Cellular casts containing erythrocytes
  - 1) **Disease association:** Bleeding within the nephron, damage to the glomerulus or renal capillaries as found in post-streptococcal infections
  - 2) Seen following strenuous contact sports
  - 3) **Appearance:** Orange to red color, contains hemoglobin and intact erythrocytes
- c. **WBC cast:** A cellular cast containing WBCs
  - 1) **Disease association:** Infection (pyelonephritis) or inflammation within the nephron (acute interstitial nephritis)
  - 2) **Appearance:** Primarily contain neutrophils, thus appearing granular with multilobed nuclei
- d. **Bacterial cast:**
  - 1) **Disease association:** Pyelonephritis
  - 2) **Appearance:** Bacilli contained within the cast and bound to the surface; mixed cast containing bacteria and WBCs may occur
- e. **Epithelial cell cast:**
  - 1) **Disease association:** Advanced renal tubular damage; seen in heavy metal, chemical, or drug toxicity, viral infections, and allograft rejection
  - 2) **Appearance:** Contain renal tubular epithelial cells
- f. **Granular cast:**
  - 1) **Disease association:** The granular appearance may result from glomerular precipitants, such as cellular casts or protein aggregates.
  - 2) Seen with hyaline casts following stress and exercise
  - 3) **Appearance:** Granular casts can be coarsely or finely granular (differentiation holds no clinical significance). **Finely granular casts** appear gray or pale yellow. **Coarsely granular casts** contain larger granules that may appear black.
- g. **Waxy cast:** Contains surface protein, granules adhere to the cast matrix; formed from degeneration of granular casts
  - 1) **Disease association:** Chronic renal failure with significant urine stasis
  - 2) **Appearance:** High refractive index, colorless to yellow with a smooth appearance; can have cracks or fissures on the sides
- h. **Fatty cast:** Seen with oval fat bodies in disease states that result in lipiduria

- 1) **Disease association:** Nephrotic syndrome, toxic tubular necrosis, diabetes mellitus
- 2) **Appearance:** Highly refractile, contains yellow-brown fat droplets
- 3) Positive identification of fatty casts is by Sudan III stain or polarized light, which shows a characteristic **Maltese cross formation**.
- i. **Broad cast:** Formed in the DCT and CD due to **anuria**
  - 1) **Disease association:** Suggests renal failure
  - 2) **Appearance:** All types of casts can occur in the broad form, with the most common being granular and waxy
6. **Bacteria:** Not present in normal sterile urine
  - a. **Disease association:** Lower and upper UTI
  - b. Bacteria can be distinguished from amorphous crystals by their motility (tumbling or directional flagellar movement).
7. **Yeast:**
  - a. **Disease association:** UTI, vaginal infections, diabetes mellitus, and in immunocompromised individuals
  - b. Yeast can be confused with erythrocytes; look for budding yeast forms.
8. **Parasites:** The most common parasite in the urine is *Trichomonas vaginalis*. Another parasite sometimes found in the urine is **pinworm ova** from *Enterobius vermicularis*, which is usually due to fecal contamination.
9. **Sperm:** Seen in urine following intercourse or nocturnal emissions; no clinical significance except in forensic cases, male infertility, and retrograde ejaculation
10. **Mucus:** Protein substance produced by the RTE cells and the urogenital glands; not considered clinically significant
  - a. **Appearance:** Threadlike structures with low refractive index, view under reduced light; can be confused with hyaline casts
11. **Crystals:**
  - a. Crystals are formed by the **precipitation** of urine salts, organic compounds, and medications. Crystal formation can be altered by temperature, pH, and urine concentration. Crystals will appear more frequently if urine stands at room temperature for prolonged time periods or is refrigerated. Urine **pH is important** in determining the **type of crystal formation**.
  - b. **Crystal formation:** The glomerular ultrafiltrate passes through the renal tubules; then the solutes in the ultrafiltrate are concentrated. If an increased amount of a solute is present, the **ultrafiltrate** becomes **saturated**, leading to the solute precipitating into a characteristic crystal form. Crystal formation is enhanced when **urine flow** through the renal tubules is **inhibited**. The reduced flow allows time for concentration of the solutes in the ultrafiltrate.
  - c. **Crystal identification:** Crystals differ in their solubility. **All clinically significant crystals are in acidic and neutral urine.**

## 12. Types of acidic urine crystals:

### a. Amorphous urates

- 1) Formed from the urate salts of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^+$ , and  $\text{Ca}^{2+}$
- 2) No clinical significance
- 3) Small, **yellow-to-brown granules** usually in large amounts; may make other urine elements difficult to see
- 4) **Refrigerated** samples will produce more **amorphous urates** and may appear as **pink sediment** because of the **presence of uroerythrin** on the surface of the granules.
- 5) Amorphous urates will **dissolve at alkaline pH** or by heating above  $60^\circ\text{C}$ .

### b. Uric acid

- 1) Seen in **gout**, with chemotherapy for leukemia, and in Lesch-Nyhan syndrome
- 2) Appear **yellow to orange/brown** but can be colorless
- 3) **Pleomorphic** (many) shapes include four-sided flat plates, rhombic plates, wedges, and rosettes

### c. Calcium oxalate

- 1) Most urine oxalate is from oxalic acid, which is found in such foods as tomatoes, asparagus, spinach, berries, and oranges. In addition, oxalic acid is a metabolite of ascorbic acid.
- 2) Colorless, **dihydrate** form appears as **octahedral envelope** or two pyramids joined at their bases; **monohydrate** form appears as **dumbbell or oval** shaped.
- 3) These crystals are **associated with renal calculi formation**. Monohydrate form is seen in poison centers where children have ingested ethylene glycol (antifreeze).

### d. Bilirubin

- 1) **Abnormal crystal:** These crystals are formed when urine bilirubin exceeds its solubility, and they appear as **fine needles or granules** that are **yellow to brown** in color.
- 2) These crystals are most often seen in **liver disease**. Casts may contain bilirubin crystals in cases of viral hepatitis when there is renal tubular damage.

### e. Tyrosine

- 1) **Abnormal crystal:** Fine delicate **needles, colorless to yellow** found in clumps or rosettes
- 2) Associated with **severe liver disease** and in **inherited diseases** that affect amino acid metabolism
- 3) May be seen with leucine crystals in urine that tests positive for bilirubin

f. **Leucine**

- 1) **Abnormal crystal: Yellow to brown**, oily-looking spheres with concentric circles and radial striations; may be found with tyrosine crystals
- 2) Associated with **severe liver disease** and in **inherited diseases** that affect amino acid metabolism

g. **Cystine**

- 1) **Abnormal crystal: Colorless, hexagonal plates**
- 2) These crystals result from a **congenital disorder** that inhibits renal tubular reabsorption of cystine, hence cystinuria. Note that lysine, arginine, and ornithine will also be present but they are more soluble than cysteine so not visible.
- 3) Associated with **renal calculi formation**

h. **Cholesterol**

- 1) **Abnormal crystal: Clear, flat, rectangular plates with a notch in one or more corners**; more commonly seen following refrigeration; seen with fatty casts and oval fat bodies
- 2) Associated with **nephrotic syndrome** and other disorders that produce lipiduria

i. **Crystals from medications**

- 1) Medications are excreted by the kidneys; any buildup can result in renal damage.
  - a) **Ampicillin** crystals appear **colorless** in the form of **needles**, which may form bundles.
  - b) **Sulfonamide** crystals appear **colorless to yellow-brown** in the form of needles, sheaves of wheat, fan formations, or rosettes.

j. **Radiographic dyes**

- 1) Resemble cholesterol
- 2) Correlate with increased specific gravity ( $>1.050$ )

13. **Alkaline urine crystals:**a. **Amorphous phosphate**

- 1) Identical in appearance to amorphous urates and generally **colorless**; **refrigerated** samples appear as a **white sediment**
- 2) Amorphous phosphates are **soluble in acetic acid** (amorphous urates are insoluble in acetic acid) and will not dissolve when heated above  $60^{\circ}\text{C}$ .
- 3) Not clinically significant

b. **Triple phosphate (ammonium magnesium phosphate)**

- 1) **Colorless, three- to six-sided prism** often resembling a **coffin lid**
- 2) Not clinically significant; may be associated with **UTI**

c. **Calcium phosphate**

- 1) **Colorless**, thin prisms or rectangular plates
- 2) Not clinically significant; may be associated with **renal calculi formation**



d. **Ammonium biurate**

- 1) Normal crystal commonly seen in old urine samples; converts to uric acid crystals if acetic acid added and dissolves at 60°C
- 2) **Yellow to brown** spheres with striation on the surface, also can show irregular, **thorny projections** (thorn apple)

e. **Calcium carbonate**

- 1) Appear as small, **colorless** crystals having **dumbbell** or spherical shapes
- 2) Not clinically significant

## VIII. SPECIAL URINE SCREENING TESTS (USUALLY PERFORMED IN SPECIAL CHEMISTRY)

### A. Phenylketonuria (PKU)

1. The presence of phenylalanine in the urine indicates defective metabolic conversion of phenylalanine to tyrosine, which is caused by a gene failure to produce **phenylalanine hydroxylase**.
2. Occurs in 1:10,000 births and, if undetected, will result in **severe mental retardation**.
3. **PKU screening tests** are required in all 50 states for newborns (at least 24 hours old).
4. The urine gives off a **mousy odor** associated with phenylpyruvate because of the increased ketones.
5. When the PKU test is positive, the diet is changed to eliminate all phenylalanine from the diet.
6. As the child grows, an alternative phenylalanine pathway develops and dietary restrictions are eased.
7. **Types of PKU testing** (all positive screening tests are **confirmed by high-performance liquid chromatography**)
  - a. **Guthrie** bacterial inhibition test uses blood from a heel stick. Blood is placed on filter paper disks on culture media streaked with *Bacillus subtilis*. If phenylalanine is present in the blood sample, beta-2-thienylalanine in the media, which is an inhibitor of *B. subtilis*, will be counteracted, resulting in *B. subtilis* growth around the disks (positive PKU).
  - b. Urine test for phenylpyruvic acid uses **ferric chloride** (tube or reagent strip); a positive test is blue-green.
    - 1) **Microfluorometric assay** directly measures phenylalanine in dried blood filter disks.
    - 2) It is a quantitative test (Guthrie is semiquantitative).
    - 3) It is not affected by antibiotics.
    - 4) A pretreated (trichloroacetic acid) patient sample extract is reacted in a microtiter plate containing ninhydrin, succinate, leucylalanine, and copper tartrate.
    - 5) The sample is measured at 360 nm and 530 nm.

## B. Miscellaneous Special Urine Screening Tests

### 1. Tyrosinosis

- Excess **tyrosine** (tyrosinuria) or its by-products (*p*-hydroxyphenylpyruvic acid or *p*-hydroxyphenyllactic acid) in the urine
- Inherited or metabolic defects
- Metabolic disease states** include transitory tyrosinemia, premature infants with an underdeveloped liver, and acquired severe liver disease. All of these conditions will produce **tyrosine and leucine crystals**. **Hereditary defects** are usually **fatal**, presenting with liver and renal diseases.
- The **nitrosonaphthol** test is used as a **screening** test, whereas **chromatography** is used as a **confirmatory** test.

### 2. Alkaptonuria

- This is a **genetic defect** resulting in failure to produce homogentisic acid oxidase, which causes **accumulation of homogentisic acid** in blood and urine.
- This condition produces brown pigment deposits in body tissue that can lead to arthritis, liver, and cardiac problems.
- Screening tests** include:
  - Ferric chloride tube test (blue color)
  - Benedict's test (yellow color)
  - Alkalization of fresh urine (urine darkens)

### 3. Melanuria

- Increased melanin** in urine is produced from **tyrosine**; urine darkens upon standing.
- Indicates **malignant melanoma**
- Screening tests include **ferric chloride** (gray/black precipitate) and **sodium nitroprusside** (red color).

### 4. Maple syrup urine disease

- Characteristic of this disorder is **maple syrup smell** of the urine, breath, and skin.
- Caused by low levels of **branched-chained keto acid decarboxylase**; inhibits metabolism of leucine, isoleucine, and valine
- If **untreated**, the disease causes **severe mental retardation**, convulsions, acidosis, and hypoglycemia. Death occurs during the first year.
- Screening test uses **2,4-dinitrophenylhydrazine (DNPH)** to form yellow turbidity or precipitate.

### 5. Argentaffinoma

- Arise from enterochromaffin cells of the gastrointestinal tract
- Produce increased blood serotonin, whose major urinary excretion product is 5-hydroxyindoleacetic acid (5-HIAA)
- Detected using 1-nitroso-2-naphthol to yield a purple color
- Patient must be on diet free of bananas, pineapples, and tomatoes, which contain significant amounts of serotonin.

## IX. BODY FLUIDS AND FECAL ANALYSIS

### A. Cerebrospinal Fluid (CSF): (Most CSF analysis is performed in chemistry, hematology, and microbiology.)

1. **CSF** is made by the brain's third choroid plexus as an ultrafiltrate of plasma. It supplies nutrients to nervous tissue, removes wastes, and cushions the brain and spinal cord against trauma. 20 mL of CSF is produced each hour. Total adult volume is 140–170 mL, and neonate volume is 10–60 mL.
2. **Specimen collection** is by lumbar puncture, which is performed by a physician between 3–4 or 4–5 lumbar vertebrae. The samples are collected into sterile, numbered, screw-capped tubes. The **order of draw** is:
  - a. Tube #1: Chemistry and serology (glucose, protein, antibodies); note that hematology will also receive this tube initially to compare with tube #3 for determining possibility of traumatic tap
  - b. Tube #2: Microbiology (culture and sensitivity)
  - c. Tube #3: Hematology (red and white blood cell counts)
3. **Cerebrospinal fluid appearance**
  - a. **Clear and colorless:** Normal
  - b. **Cloudy:** Indicates WBCs, RBCs, protein, or bacteria; seen in meningitis, hemorrhage, disorders of the blood-brain barrier, etc.
  - c. **Bloody:** This may be due to subarachnoid hemorrhage or traumatic tap. Differentiation between the two is made by noting the difference in appearance between tubes #1 and #3. If there is hemorrhage, all tubes will appear bloody. If traumatic tap, there will be less blood in tube #3 than in tube #1.
  - d. **Xanthochromic** (yellow): Increased hemoglobin, bilirubin, protein, immature liver in premature infants
4. **Chemistry testing**
  - a. **CSF glucose:** 60–70% of the patient's plasma glucose
  - b. **CSF total protein:** Assayed using trichloroacetic acid precipitation method or Coomassie brilliant blue
5. **Hematology testing**
  - a. **Cerebrospinal fluid microscopic:** Normal CSF contains 0–5 WBC/ $\mu$ L; lymphocytes and monocytes predominate
  - b. **Lymphocytes:** Seen in normal fluids; **increased in viral and fungal meningitis**
  - c. **PMNs: Bacterial meningitis** (cerebral abscess)
  - d. **Early cell forms:** Acute leukemia
  - e. **Plasma cells:** Multiple sclerosis or lymphocytic reactions
6. **Microbiology testing**
  - a. **India ink:** Used to detect *Cryptococcus neoformans*, possible complication of AIDS
  - b. **Gram stain and culture:** Used to detect bacteria

## B. Seminal Fluid (Semen)

1. Used to evaluate infertility, post-vasectomy, and forensic medicine cases
2. **Specimen collection**
  - a. Collect in sterile containers after 3-day period of no sexual activity for infertility studies; **post-vasectomy** requires no waiting period
  - b. Plastic containers will inhibit motility
  - c. No condom collection (may contain spermicidal agents)
  - d. Keep at room temperature; transport to the lab professionals within 1 hour.
3. **Semen analysis**
  - a. **Volume:** 2–5 mL
  - b. **Viscosity:** Normal is no clumps or strings; must have specimen that is completely liquefied, which takes about 30–60 minutes.
  - c. **Appearance:** Normal is a **translucent, gray-white color**.
  - d. **pH:** 7.2–8.0 is normal (>8.0 could indicate infection).
  - e. **Sperm count:** Normal is 20–160 million/mL, borderline is 10–20 million/mL, and sterile is less than 10 million/mL.
  - f. **Motility:** Based on the percentage of movement, 50–60% or greater with a motility grade of 2 is normal (0—immotile; 4—motile with strong forward progression).
  - g. **Morphology: Oval-shaped head with a long, flagellar tail is normal.** **Abnormal** forms include double head, giant head, amorphous head, pinhead, double tail, and coiled tail.
  - h. **Cells other than sperm present:** The presence of red blood cells or white blood cells would be significant.

## C. Synovial Fluid

1. Synovial fluid is a **plasma ultrafiltrate** and is often called **joint fluid**.
2. Synovial fluid functions as a **lubricant** and **nutrient transport** to articular cartilage.
3. Different joint disorders change the chemical and structural composition of synovial fluid, including inflammation, infection, bleeding, and crystal-associated disorders.
4. Normal **color** of synovial fluid is **clear to straw** colored.
5. **Laboratory analysis** (nonchemistry) includes color, differential count, Gram stain with culture, and crystal identification with a polarizing microscope.
  - a. In addition, the presence of hyaluronic acid gives synovial fluid a unique viscosity, which, if absent, can suggest the presence of bacteria secreting hyaluronidase.
  - b. Most **crystals** found in synovial fluid are associated with **gout (uric acid)** or **calcium phosphate deposits**.

**D. Gastric Fluid:** Gastric fluid collection is performed by nasal or oral intubation. Analysis involves physical appearance, volume, titratable acidity, and pH. Most uses of gastric analysis are for toxicology and for the diagnosis of Zollinger-Ellison syndrome.

#### E. Amniotic Fluid

1. **Protective fluid** surrounding the fetus; needle aspiration termed **amniocentesis**
2. Amniotic fluid is mostly used for genetic studies but may be used to check for bilirubin, fetal bleeding, infection, fetal lung maturity, or **meconium** (dark green fetal intestinal secretions) content that in large amounts is associated with meconium aspiration syndrome.
3. Differentiation of the presence of blood versus bilirubin can be achieved by measuring for increased absorbance at 410 nm (bilirubin) and 450 nm (hemoglobin).
4. Levels of phospholipids (phosphatidyl choline [lecithin], phosphatidyl glycerol) will increase as the fetus's lungs mature.

#### F. Peritoneal Fluid

1. **Clear to pale yellow** fluid contained between the parietal and visceral membranes in the **peritoneum** (serous membrane that covers the walls of the abdomen and pelvis); also called **ascites fluid**
2. Aspiration is termed **peritoneocentesis**.
3. **Laboratory analysis** includes cell counts, Gram stains, gross color examination and specific gravity.

#### G. Pleural and Pericardial Fluids

1. Pleural and pericardial fluids are found between the visceral and parietal pleural (around the lungs) and pericardial (around the heart) membranes, respectively. Both fluids are **clear to pale yellow**.
2. Aspiration of pleural/pericardial fluids is termed **thoracentesis** and **pericardiocentesis**, respectively.
3. **Laboratory analysis** includes cell counts, Gram stains, and gross color examination.

#### H. Fluid Effusions

1. Increases in volume in peritoneal, pleural, or pericardial fluids are called **effusions**.
2. If the mechanism is **noninflammatory**, it is called a **transudate** and will have **fewer than 1000 cells/ $\mu$ L and less than 3 g/dL protein**.
3. **Inflammatory** effusions are called **exudates** and will have **higher than 1000 cells/ $\mu$ L and more than 3 g/dL protein**.

## I. Fecal Analysis

1. Used in the detection of gastrointestinal (GI) bleeding, liver and biliary duct disorders, malabsorption syndromes, and infections
2. Types of fecal analysis
  - a. **Color and consistency**
    - 1) **Black (tarry) stool:** Upper GI bleeding; iron therapy
    - 2) **Red stool:** Lower GI bleeding
    - 3) **Steatorrhea:** Fat malabsorption
    - 4) **Diarrhea:** Watery fecal material
    - 5) **Ribbon-like stools:** Bowel obstruction
    - 6) **Mucus:** Inflammation of the intestinal wall (colitis)
    - 7) **Clay-colored, pale:** Bile-duct obstruction/obstructive jaundice
  - b. **Fecal leukocytes:** Determine cause of diarrhea
    - 1) **Neutrophils:** Bacterial intestinal wall infections or ulcerative colitis, abscesses
    - 2) **No neutrophils:** Toxin-producing bacteria, viruses, and parasites
  - c. **Qualitative fecal fat:** Detects fat malabsorption disorders by staining fecal fats with **Sudan III or oil red O**; increased fecal fat (>60 droplets/hpf) suggestive of **steatorrhea**
  - d. **Muscle fibers:** Look for undigested striated muscle fibers, which may indicate **pancreatic insufficiency** seen in cystic fibrosis.
  - e. **Occult blood:** Used for early detection of **colorectal cancer**; old name, guaiac test
    - 1) Occult blood most frequently performed fecal analysis
    - 2) Several chemicals used that vary in sensitivity
      - a) **Ortho-toluidine:** Pseudoperoxidase activity of hemoglobin (Hb) reacts with  $\text{H}_2\text{O}_2$  to oxidize a colorless reagent to a colored product.  
  

$$\text{Hb} \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{ortho-toluidine} \rightarrow \text{blue oxidized indicator}$$
      - b) **Gum guaiac:** Least sensitive, most common
      - c) Immunological: Use of an antihemoglobin to react with the patient's hemoglobin has the advantage of not requiring any special diet before sample collection. There is the possibility, however, of hemoglobin degradation (and nondetection by antibody), if the gastrointestinal bleed is in the upper intestine.
  - f. **DNA test** detects **K-ras mutation**, which is associated with colorectal cancer.



# review questions

## INSTRUCTIONS

Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

1. Why is the first-voided morning urine specimen the most desirable specimen for routine urinalysis?
  - A. Most dilute specimen of the day and therefore any chemical compounds present will not exceed the detectability limits of the reagent strips
  - B. Least likely to be contaminated with microorganisms because the bladder is a sterile environment
  - C. Most likely to contain protein because the patient has been in the orthostatic position during the night
  - D. Most concentrated specimen of the day and therefore it is more likely that abnormalities will be detected
2. The physical characteristic of color is assessed when a routine urinalysis is performed. What substance is normally found in urine that is principally responsible for its yellow coloration?
  - A. Bilirubin
  - B. Melanin
  - C. Carotene
  - D. Urochrome
3. In certain malignant disorders, what substance is found in the urine that turns the urine dark brown or black on exposure of the urine to air?
  - A. Urobilinogen
  - B. Indican
  - C. Melanin
  - D. Porphyrin
4. What is the expected pH range of a freshly voided urine specimen?
  - A. 3.5–8.0
  - B. 3.5–9.0
  - C. 4.0–8.5
  - D. 4.5–8.0



5. Urine specimens should be analyzed as soon as possible after collection. If urine specimens are allowed to stand at room temperature for an excessive amount of time, the urine pH will become alkaline because of bacterial decomposition of
  - A. Protein
  - B. Urea
  - C. Creatinine
  - D. Ketones
6. Which term is defined as a urine volume in excess of 2000 mL excreted over a 24-hour period?
  - A. Anuria
  - B. Oliguria
  - C. Polyuria
  - D. Hypersthenuria
7. The reagent test strips used for the detection of protein in urine are most reactive to
  - A. Albumin
  - B. Hemoglobin
  - C. Alpha-globulins
  - D. Beta-globulins
8. A urine specimen that exhibits yellow foam on being shaken should be suspected of having an increased concentration of
  - A. Protein
  - B. Hemoglobin
  - C. Bilirubin
  - D. Nitrite
9. How should controls be run to ensure the precision and accuracy of the reagent test strips used for the chemical analysis of urine?
  - A. Positive controls should be run on a daily basis and negative controls when opening a new bottle of test strips.
  - B. Positive and negative controls should be run when the test strips' expiration date is passed.
  - C. Positive and negative controls should be run on a daily basis.
  - D. Positive controls should be run on a daily basis and negative controls on a weekly basis.
10. The colorimetric reagent strip test for protein is able to detect as little as 5–20 mg of protein per deciliter. What may cause a false-positive urine protein reading?
  - A. Uric acid concentration is greater than 0.5 g/day.
  - B. Vitamin C concentration is greater than 0.5 g/day.
  - C. Glucose concentration is greater than 130 mg/day.
  - D. pH is greater than 8.0.
11. "Isosthenuria" is a term applied to a series of urine specimens from the same patient that exhibit a
  - A. Specific gravity of exactly 1.000
  - B. Specific gravity less than 1.007
  - C. Specific gravity greater than 1.020
  - D. Fixed specific gravity of approximately 1.010

12. A urine specimen is tested by a reagent strip test and the sulfosalicylic acid test to determine whether protein is present. The former yields a negative protein, whereas the latter results in a reading of 2+ protein. Which of the following statements best explains this difference?
- A. The urine contained an excessive amount of amorphous urates or phosphates that caused the turbidity seen with the sulfosalicylic acid test.
  - B. The urine pH was greater than 8, exceeding the buffering capacity of the reagent strip, thus causing a false-negative reaction.
  - C. A protein other than albumin must be present in the urine.
  - D. The reading time of the reagent strip test was exceeded (the reading being taken at 2 minutes), causing a false-negative reaction to be detected.
13. Which of the following is the major organic substance found in urine?
- A. Sodium
  - B. Potassium
  - C. Glucose
  - D. Urea
14. Each of the following is included in the quality assurance program for a urinalysis laboratory. Which one represents a preanalytical component of testing?
- A. Setting collection guidelines for 24-hour urines
  - B. Setting a maintenance schedule for microscopes
  - C. Reporting units to be used for crystals
  - D. Requiring acceptable results for control specimens before any patient results are reported out
15. The presence of ketone bodies in urine specimens may be detected by use of a reagent strip impregnated with sodium nitroprusside. This strip test is sensitive to the presence of
- A. Acetoacetic acid and beta-hydroxybutyric acid
  - B. Acetoacetic acid and acetone
  - C. Diacetic acid and beta-hydroxybutyric acid
  - D. Beta-hydroxybutyric acid and acetone
16. A routine urinalysis is performed on a young child suffering from diarrhea. The reagent test strip is negative for glucose but positive for ketones. These results may be explained by which of the following statements?
- A. The child has Type 1 diabetes mellitus.
  - B. The child is suffering from lactic acidosis, and the lactic acid has falsely reacted with the impregnated reagent area for ketones.
  - C. The child is suffering from increased catabolism of fat because of decreased intestinal absorption.
  - D. The reagent area for ketones was read after the maximum reading time allowed.
17. The principle of the colorimetric reagent strip test for hemoglobin is based on the peroxidase activity of hemoglobin in catalyzing the oxidation of a dye with peroxide to form a colored compound. This method may yield false-positive results for the presence of hemoglobin when the urine specimen contains
- A. Ascorbic acid
  - B. Tetracycline
  - C. Myoglobin
  - D. Nitrite

18. A reagent test strip impregnated with a diazonium salt such as diazotized 2,4-dichloroaniline may be used to determine which analyte?
  - A. Glucose
  - B. Ketone
  - C. Hemoglobin
  - D. Bilirubin
19. Which of the following will contribute to a specimen's specific gravity if it is present in a person's urine?
  - A. 50–100 RBC/hpf
  - B. 85 mg/dL glucose
  - C. 3+ amorphous phosphates
  - D. Moderate bacteria
20. With infections of the urinary system, white blood cells are frequently seen in the urine sediment. What type of white blood cell is seen the most frequently in urine sediment?
  - A. Eosinophil
  - B. Lymphocyte
  - C. Monocyte
  - D. Neutrophil
21. A random urine is collected from a patient and the results obtained are as follows: urine albumin = 16 mg/dL and urine creatinine = 140 mg/dL. These findings are consistent with
  - A. Microalbuminuria
  - B. Macroalbuminuria
  - C. Nephrotic syndrome
  - D. Obstructive jaundice
22. To detect more easily the presence of casts in urine sediments, which microscopic method can be used?
  - A. Fluorescent microscopy
  - B. Phase-contrast microscopy
  - C. Polarized microscopy
  - D. Brightfield microscopy
23. Which substance found in urinary sediment is more easily distinguished by use of polarized microscopy?
  - A. Lipids
  - B. Casts
  - C. Red blood cells
  - D. Ketone bodies
24. "Glitter cell" is a term used to describe a specific type of
  - A. Ketone body
  - B. Oval fat body
  - C. Fatty droplet
  - D. Neutrophil
25. The final phase of degeneration that granular casts undergo is represented by which of the following casts?
  - A. Fine
  - B. Coarse
  - C. Cellular
  - D. Waxy
26. A 40-year-old female patient with a history of kidney infection is seen by her physician because she has felt lethargic for a few weeks. She has decreased frequency of urination and a bloated feeling. Physical examination shows periorbital swelling and general edema, including a swollen abdomen. Significant urinalysis results show the following: color = yellow; appearance = cloudy/frothy; specific gravity = 1.022; pH = 7.0; protein = 4+; 0–3 WBC/hpf; 0–1 RBC/hpf; 0–2 renal epithelial cells/hpf; 10–20 hyaline casts/lpf; 0–1 granular casts/lpf; 0–1 fatty casts/lpf; occasional oval fat bodies. Her serum chemistries show significantly decreased albumin, increased urea nitrogen, and increased creatinine. These findings suggest which condition?
  - A. Multiple myeloma
  - B. Glomerulonephritis
  - C. Nephrotic syndrome
  - D. Chronic renal failure

27. A 47-year-old female patient with controlled type 2 diabetes mellitus complains of urinary frequency and burning. She provides a first-morning, clean-catch specimen. Results show color = yellow; appearance = cloudy; pH = 6.5; a representative microscopic high-power field is shown in Color Plate 46■. Which of the following is *true* for this patient?
- The number of bacteria seen would result in a positive nitrite.
  - The major formed elements are white blood cells and yeast.
  - The type and number of epithelial cells suggest incorrect sample collection.
  - The red blood cells would be sufficient to give a positive blood result on the reagent strip.
28. Alkaptonuria, a rare hereditary disease, is characterized by the urinary excretion of
- Alkaptone
  - Phenylalanine
  - 5-Hydroxyindole acetic acid
  - Homogentisic acid
29. A 22-year-old female clinical laboratory student performs a urinalysis on her own urine as part of a lab class. Significant results include: color = yellow; appearance = cloudy; pH = 7.5; nitrite = positive; leukocyte esterase = 2+; 25–40 WBC/hpf; 0–3 RBC/hpf; 2–5 squamous epithelial cells/hpf; moderate bacteria. All other chemistries and microscopic results were normal. These findings suggest
- Glomerulonephritis
  - Upper urinary tract infection
  - Lower urinary tract infection
  - Nephrolithiasis
30. Metastatic carcinoid tumors arising from the enterochromaffin cells of the gastrointestinal tract are characterized by increased excretion of urinary
- Serotonin
  - 5-Hydroxytryptophan
  - Homogentisic acid
  - 5-Hydroxyindole acetic acid
31. Some clinical conditions are characterized by unique urinalysis result patterns. Which of the following shows such a relationship?
- Nephrotic syndrome: positive protein on reagent strip, negative protein with sulfosalicylic acid
  - Intensive dieting: increased ketones, negative glucose
  - Multiple myeloma: positive protein by both reagent strip and sulfosalicylic acid
  - Cystitis: positive nitrite and protein
32. Nitrite in a urine specimen suggests the presence of
- White blood cells
  - Red blood cells
  - Bacteria
  - Yeasts
33. If a fasting plasma glucose level of 100 mg/dL is obtained on an individual, what is the expected fasting cerebrospinal fluid (CSF) glucose level in mg/dL?
- 25
  - 50
  - 65
  - 100

34. A 35-year-old man has just experienced severe crush injuries sustained in a car accident. He has a broken pelvis and right femur and has numerous abrasions and contusions. A random urinalysis specimen shows a brown color and clear appearance. pH is 6.0, protein is 1+, and blood is 3+. There is, however, only 0–1 RBC/hpf, along with 0–3 WBC/hpf. Casts found include hyaline (0–2/lpf) and granular (0–1/lpf). Other urine results are normal. Which of the following is *true* about this patient?
- A. The positive blood result is from a hemolytic anemia.
  - B. The bilirubin result should have also been positive for this patient.
  - C. Rhabdomyolysis may be a cause for the discrepant chemical/microscopic blood findings.
  - D. The bone crushing led to the increased protein result.
35. A 67-year-old male has routine testing done and shows an estimated glomerular filtration rate (eGFR) of 42 mL/min/1.73 m<sup>2</sup>. Which of the following is *true* for this patient?
- A. This test requires a 24-hour urine collection.
  - B. The patient does not have chronic kidney damage, based on these results.
  - C. Similar results would be obtained using the Cockcroft-Gault formula.
  - D. The patient is in Stage 3 chronic kidney damage.
36. Which is *true* about the formed element shown in Color Plate 47■?
- A. May be found in normal alkaline urine
  - B. Associated with renal pathology
  - C. Characteristic of glomerulonephritis
  - D. Associated with lung pathology
37. The major formed element in the high-power field shown in Color Plate 48■ is most likely a
- A. Granular cast
  - B. Hyaline cast
  - C. Waxy cast
  - D. Fiber artifact
38. Which of the following is *true* about the final concentrating of urine in the kidney?
- A. The distal convoluted tubule, through active transport, reabsorbs water.
  - B. Water is reabsorbed under the direct influence of angiotensin II.
  - C. Vasopressin controls the collecting duct reabsorption of water.
  - D. Water reabsorption is influenced by urine filtrate levels of potassium.
39. If a urine specimen is left standing at room temperature for several hours, which of the following changes may occur?
- A. Multiplication of bacteria
  - B. An increase in the glucose concentration
  - C. Production of an acid urine
  - D. Deterioration of any albumin present
40. The formed element shown in Color Plate 49■ would usually be found in the patient's urine along with which soluble biochemicals?
- A. Phenylalanine and tyrosine
  - B. Ornithine and arginine
  - C. Isoleucine and leucine
  - D. Acetoacetic acid and  $\beta$ -hydroxybutyric acid

41. A 13-year-old ice skater is having her routine physical before the school year. Her first morning urinalysis results include color = straw; appearance = hazy; pH = 6.0; protein = trace; a representative microscopic high-power field is shown in Color Plate 50■. All other chemical results were normal. The major formed elements are \_\_\_\_\_ and suggest \_\_\_\_\_.
  - A. Hyaline casts and waxy casts; nephrotic syndrome
  - B. Mucus and fibers; no pathology
  - C. Granular casts and red blood cells; glomerulonephritis
  - D. Hyaline casts and mucus; normal sediment
42. Phenylketonuria may be characterized by which of the following statements?
  - A. It may cause brain damage if untreated.
  - B. It is caused by the absence of the enzyme, phenylalanine oxidase.
  - C. Phenylpyruvic acid excess appears in the blood.
  - D. Excess tyrosine accumulates in the blood.
43. What condition is suggested by the number of the formed element that predominates in the high-power field of Color Plate 51■?
  - A. Glomerulonephritis
  - B. Improperly collected specimen
  - C. Pyelonephritis
  - D. Normal sample
44. Xanthochromia of cerebrospinal fluid (CSF) samples may be due to increased levels of which of the following?
  - A. Chloride
  - B. Protein
  - C. Glucose
  - D. Magnesium
45. Which of the following will be characterized by an increased number of the urinary component seen in Color Plate 52■?
  - A. Acute glomerulonephritis
  - B. Biliary tract obstruction
  - C. Contamination from vaginal discharge
  - D. Nephrotic syndrome
46. To determine amniotic fluid contamination with maternal urine, which of the following measurements could be used?
  - A. Creatinine concentration
  - B. Delta absorbance at 410 nm
  - C. Albumin/globulin ratio
  - D. Lactate dehydrogenase
47. With the development of fetal lung maturity, which of the following phospholipid concentrations in amniotic fluid significantly and consistently increases?
  - A. Sphingomyelin
  - B. Phosphatidyl ethanolamine
  - C. Phosphatidyl inositol
  - D. Phosphatidyl choline
48. A patient has been diagnosed with an upper gastrointestinal bleed. Which of the following would be characteristic for this condition?
  - A. Brown stool with streaks of bright red
  - B. Stool with lack of brown color ("clay-colored")
  - C. Stool with a much darker brown/black color
  - D. Yellow stool with increased mucus
49. A pleural effusion is found to have 3000 white blood cells per microliter and 5 g/dL total protein. From this it can be determined that the patient's effusion is
  - A. A transudate
  - B. An exudate
  - C. Noninflammatory
  - D. Hemorrhagic



50. Patients with diabetes insipidus tend to produce urine in \_\_\_\_\_ volume with \_\_\_\_\_ specific gravity.
- Increased; decreased
  - Increased; increased
  - Decreased; decreased
  - Decreased; increased
51. The estimation of hyaluronic acid concentration by measurement of viscosity is useful in evaluating which type of fluid?
- Spinal
  - Peritoneal
  - Pleural
  - Synovial
52. Which of the following is characteristic of an exudate effusion?
- Leukocyte count  $>1000/\mu\text{L}$
  - Clear appearance
  - Protein concentration  $<3.0\text{ g/dL}$
  - Absence of fibrinogen
53. Which of the following systems utilizes polyelectrolytes to determine the specific gravity of urine?
- Refractometer
  - Osmometer
  - TS meter
  - Reagent strip
54. Which methods may be used to quantify protein in both cerebrospinal fluid and urine specimens?
- Trichloroacetic acid and bromocresol green
  - Ponceau S and Coomassie brilliant blue
  - Bromocresol green and Coomassie brilliant blue
  - Coomassie brilliant blue and trichloroacetic acid
55. Which of the following characteristics is *true* of the primary urinary components shown in Color Plate 53■?
- Consist of uromodulin protein
  - Presence always indicates a disease process
  - Can be observed with polarized microscopy
  - Appear yellowish in brightfield microscopy
56. A characteristic of substances normally found dissolved in the urine is that they are all
- Water soluble
  - Inorganic
  - Organic
  - Waste products
57. Which of the following statements applies to the proper collection and handling of CSF?
- The second tube collected should be used for chemistry analyses.
  - The third tube collected should be used for bacteriologic studies.
  - CSF collected in the evening should be refrigerated and assays performed only by day-shift personnel.
  - With low-volume specimens, a culture is performed first, before cell counts are done.
58. Which of the following characteristics is *true* for the urinary components shown in Color Plate 54■?
- Never should appear in a freshly collected sample
  - Can also resemble cysteine crystals
  - Appear insoluble in alkaline urine
  - Presence indicates an inborn error of metabolism



59. A patient sends the following question to an online consumer health Web site: "I am a 22-year-old female who experienced increasing headaches, thirst, and decreasing energy. I was studying in the library when I felt lightheaded and passed out. I was taken to a hospital emergency department and they told me that my serum Acetest® was 40 mg/dL and urine glucose was 500 mg/dL. What does this mean?" How would you reply?
- Your lab results pattern suggests diabetes mellitus.
  - You probably have been crash dieting recently.
  - The two results do not fit any disease pattern.
  - The tests need to be repeated because they could not possibly occur together.
60. Which urinalysis reagent strip test will never be reported out as "negative"?
- Protein
  - Urobilinogen
  - Bilirubin
  - Nitrite
61. The following urinalysis results were obtained on a 40-year-old white male whose skin appeared yellowish during the clinical examination. Color and clarity—dark brown, clear; protein—negative; glucose—negative; blood—negative; ketones—negative; bilirubin—moderate; urobilinogen—0.2 mg/dL. These results are clinically significant in which of the following conditions?
- Bile duct obstruction
  - Cirrhosis
  - Hepatitis
  - Hemolytic anemia
62. Compared to the fecal occult blood test, which of the following is a disadvantage of performing a DNA-based test to detect colon cancer?
- The DNA test is more invasive.
  - The DNA test is less sensitive.
  - The DNA test is more expensive.
  - Additional diet restrictions are needed for the DNA test.
63. Which of the following may be associated with morphologic examination of spermatozoa?
- Evaluation should include assessment of 1000 spermatozoa.
  - A small number of sperm should have normal morphologic characteristics.
  - Papanicolaou stain may be used.
  - Presence of red or white cells and epithelial cells need not be noted.
64. Which condition is characterized by increased levels of immunoglobulins in the cerebrospinal fluid, originating from within the central nervous system and not from the general blood circulation?
- Gout
  - Erythroblastosis fetalis
  - Multiple myeloma
  - Multiple sclerosis
65. Which of the following statements pertains to screening methods used to determine pregnancy?
- Immunoassays will use reagent anti-hCG to react with patient hCG.
  - A random urine specimen is the preferred specimen for pregnancy screening tests.
  - Internal controls provided within the kit will assess if the patient's specimen was collected correctly.
  - External quality control is not needed with these methods.

66. The following urinalysis biochemical results were obtained from a 4-month-old infant who experienced vomiting and diarrhea after milk ingestion and failed to gain weight: pH—6; protein—negative; glucose—negative; ketone—negative; bilirubin—negative; Clinitest®—2+. These results are clinically significant in which of the following disorders?
- A. Diabetes mellitus
  - B. Ketosis
  - C. Starvation
  - D. Galactosemia
67. Which of the following is a *true* statement?
- A. Renal tubular cells originate from the renal pelvis.
  - B. Red blood cells in acid urine (pH 4.5) will usually be crenated because of the acidity.
  - C. Bacteria introduced into a urine specimen at the time of the collection will have no immediate effect on the level of nitrite in the specimen.
  - D. Pilocarpine iontophoresis is the method of choice for the collection of pericardial fluid.



# answers & rationales

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1.

**D.** The first-voided morning urine specimen is the most desirable for chemical and microscopic analysis because it is the most concentrated specimen of the day. Protein and nitrite testing is better performed on a concentrated specimen, as are the specific gravity determination and the examination of urinary sediment. However, because of the lack of food and fluid intake during the night, glucose metabolism may be better assessed on the basis of a postprandial specimen.

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2.

**D.** Urochrome, a yellow-brown pigment derived from urobilin, is principally responsible for the yellow coloration of normal urine. Urochrome is excreted at a constant rate, showing no diurnal variation. Therefore, the color of normal urine, which may range from straw to deep amber, is dependent on the concentrating ability of the kidney and the volume of urine excreted.

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3.

**C.** Melanin, a substance derived from tyrosine, is responsible for the pigmentation of the eyes, skin, and hair. In some malignancies, known as melanomas, the tumor or mole takes on a

darkly pigmented appearance because of the melanin present. In cases of metastatic melanoma, melanogen, which is a colorless precursor of melanin, is excreted in the urine. If the urine is allowed to stand at room temperature for 24 hours, the melanogen is oxidized to melanin, imparting a dark brown or black coloration to the specimen. Qualitative screening tests for the detection of melanin in urine use ferric chloride or sodium nitroprusside as the oxidation reagent systems.

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4.

**D.** pH is a representative symbol for the hydrogen ion concentration. The kidney plays an important role in the maintenance of the acid-base balance of body fluids by either excreting or retaining hydrogen ions. A normally functioning kidney will excrete urine with a pH between 4.5 and 8.0, depending on the overall acid-base needs of the body.

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5.

**B.** At room temperature, the amount of bacteria present in a urine sample will increase. The bacteria are capable of metabolizing the urinary urea to ammonia. The ammonia formed through this process will cause an alkalization of the urine.

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6.

C. On the average, a normal adult excretes 1200–1500 mL of urine daily. “Polyuria” is a term used to describe the excretion of a urine volume in excess of 2000 mL/day. In oliguria, the daily urine excretion is less than 500 mL, and in anuria the urine formation is completely suppressed. Hypersthenuria refers to urines of any volume containing increased levels of dissolved solute.

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7.

A. In healthy individuals the amount of protein excreted in the urine should not exceed 150 mg/24 hr. When protein is present in the urine, the colorimetric reagent test strips change color, indicating a semiquantification of the amount of protein present. Serum proteins are classified as being albumin or globulin in nature, and the type of protein excreted in the urine is dependent on the disorder present. Although the strip test is a rapid screening method for the detection of urinary protein, it must be noted that this method is more sensitive to the presence of albumin in the specimen than to the presence of globulin, Bence Jones protein, or mucoprotein.

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8.

C. Normal urine does not foam on being shaken. However, urine containing bilirubin will exhibit yellow foaming when the specimen is shaken. In fact, the foam test was actually the first test for bilirubin, before the development of the chemical tests. If the shaken specimen shows a white foam, increased urine protein can be suspected.

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9.

C. For quality control of reagent test strips, it is recommended that both positive and negative controls be used daily. It is necessary that any deterioration of the strips be detected in order to avoid false-positive or false-negative results.

The use of positive and negative controls will act as a check on the reagents, on the technique employed, and on the interpretive ability of the person or instrument performing the test.

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10.

D. The principle of the reagent strip method for the detection of protein in urine is based on a color change in an indicator system, such as tetrabromophenol blue, that is buffered to pH 3. The buffering capacity of the strip is sufficient provided that the urine pH does not exceed 8.0. Within the normal urine pH range of 4.5–8.0, a change in color in the reagent strip is an indication of the presence of protein in the urine. With a urine pH greater than 8, the buffering capacity of the strip may be exceeded, and a false-positive color change in the impregnated area will reflect the pH of the urine rather than the presence of protein. The presence of vitamin C, uric acid, or glucose in urine will not affect the test for protein.

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11.

D. “Isosthenuria” is a term applied to a series of urine specimens that exhibit a fixed specific gravity of approximately 1.010. In isosthenuria there is little, if any, variation of the specific gravity between urine specimens from the same patient. This condition is abnormal and denotes the presence of severe renal damage in which both the diluting ability and the concentrating ability of the kidneys have been severely affected.

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**12.**

**C.** When globulin, mucoprotein, or Bence Jones protein is present in a urine specimen, the reagent strip test may give a negative result because the strip is more sensitive to the presence of albumin than to the presence of other proteins in urine. However, the sulfosalicylic acid (SSA) test is able to detect not only albumin but also globulin, mucoprotein, and Bence Jones protein in a specimen. Therefore, it can be seen that a negative reagent strip test result for protein but a positive sulfosalicylic acid test result is possible when the protein present is some protein other than albumin. For this reason the sulfosalicylic acid test is run as a test for urinary protein if the presence of abnormal proteins is suspected.

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**13.**

**D.** Although sodium is the major inorganic molecule found in urine, urea is the major organic molecule excreted. Urea is a waste product of protein/amino acid metabolism. Its level in a normal 24-hour urine with a glomerular filtration rate of 125 mL/min would be 400 mmol/day. Glucose excretion will average less than 1 mmol/day. The excretion of the inorganic molecules sodium and potassium would be 130 and 70 mmol/day, respectively.

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**14.**

**A.** Preanalytical components of laboratory testing include all variables that can affect the integrity or acceptability of the patient specimen prior to analysis, such as correct collection technique. Analytical factors affect the actual analysis of the specimen (temperature, condition of equipment, timing, presence of interfering substances). Postanalytical factors affect the final handling of the results generated (reporting units, critical values, acceptability of quality control).

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**15.**

**B.** Under normal metabolic conditions, the body metabolizes fat to carbon dioxide and water. With inadequate carbohydrate intake, as with dieting and starvation, or with inadequate carbohydrate metabolism, as with diabetes mellitus, there is an increased utilization of fat. Because of this increased fat metabolism, the body is unable to completely degrade the fat, resulting in a buildup of intermediary products known as ketone bodies. The term “ketone bodies” is used collectively to denote the presence of acetoacetic acid, beta-hydroxybutyric acid, and acetone. Reagent test strips impregnated with sodium nitroprusside are able to detect the presence of acetoacetic acid and acetone in urine specimens. Although beta-hydroxybutyric acid accounts for approximately 78% of the total ketones, it is not detected by the sodium nitroprusside test.

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**16.**

**C.** Although a positive result on a urine test for ketones is most commonly associated with increased urinary glucose levels, as in diabetes mellitus, other conditions may cause the urine ketone test to show positive results while the urine glucose test shows negative results. In young children, a negative glucose reaction accompanied by a positive ketone reaction is sometimes seen. Ketones in the urine may be seen when a child is suffering from an acute febrile disease or toxic condition that is accompanied by vomiting or diarrhea. In these cases, because of either decreased food intake or decreased intestinal absorption, fat catabolism is increased to such an extent that the intermediary products, known as ketone bodies, are formed and excreted in the urine.



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17.

**C.** The colorimetric reagent strip test for the detection of hemoglobin in urine utilizes a buffered test zone impregnated with a dye and organic peroxide. The peroxidase activity of hemoglobin catalyzes the oxidation of the dye with peroxide to form a colored compound. Like hemoglobin, myoglobin also has a peroxidase activity and, when present in a urine specimen, myoglobin will react, yielding false-positive results. In the presence of large amounts of ascorbic acid, antibiotics containing ascorbic acid as a preservative, formaldehyde, or nitrite, the urine reaction may be inhibited, causing false-negative results.

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18.

**D.** Bilirubin is a compound that is formed as a result of hemoglobin breakdown. The majority of bilirubin in the blood is bound to albumin and is known as unconjugated bilirubin. Because unconjugated bilirubin is not water soluble, it may not be excreted in the urine. The remainder of the bilirubin in the blood has been processed by the liver. In the liver, the bilirubin is conjugated with glucuronic acid or sulfuric acid. This conjugated bilirubin is water soluble, and it is this portion that is excreted in increased amounts in the urine in some hepatic and obstructive biliary tract diseases. The presence of conjugated bilirubin in a urine specimen may be detected by use of the reagent test strips. The test strips are impregnated with a diazonium salt, such as diazotized 2,4-dichloroaniline, which forms a purplish azobilirubin compound with bilirubin.

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19.

**B.** Only dissolved solutes affect specific gravity (e.g., glucose). Cells, mucus, crystals, or any other formed elements will have no effect, regardless of concentration. If the reagent strip method

is used, it should be noted that only dissolved ions will contribute to specific gravity results. Thus glucose would not affect reagent strip results at any concentration. In such instances as diabetes mellitus, with urine glucose levels over 2 g/dL, there may be a discrepancy between specific gravity results obtained with a reagent strip method versus using a refractometer, because such glucose levels are known to increase refractometer results, thus requiring correction.

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20.

**D.** The majority of renal and urinary tract diseases are characterized by an increased number of neutrophilic leukocytes in the urine. To identify correctly any white blood cells present in a urine specimen, it is necessary to examine the specimen as soon as possible after collection. This is necessary because leukocytes tend to lyse easily when exposed to either hypotonic or alkaline urine.

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21.

**A.** The ratio of urine albumin to creatinine in a random specimen is commonly used to evaluate microalbuminuria, especially in patients with diabetes mellitus. This patient's ratio is 114 mg albumin per gram creatinine. The American Diabetes Association defines microalbuminuria as between 30 and 299 mg/g. Values greater than 299 mg/g would be "macroalbuminuria." Nephrotic syndrome is characterized by excretion of albumin in excess of 3.5 grams per day. Patients with obstructive jaundice will usually not experience proteinuria.

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**22.**

**B.** To better diagnose renal and urinary tract diseases, it is necessary to examine urinary sediment carefully by the most appropriate microscopic method available. Formed elements in the urine, such as cells and casts, are more easily differentiated by the use of phase-contrast microscopy. This is especially true for the identification of the more translucent elements such as the hyaline casts. Phase microscopy tends to enhance the outline of the formed elements, allowing them to stand out and be more easily distinguished.

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**23.**

**A.** Fatty materials in urinary sediment may be identified by means of staining techniques using Sudan III and oil red O or by means of polarized microscopy. Polarized microscopy is especially useful when the composition of fatty casts, fatty droplets, or oval fat bodies is primarily cholesterol. When cholesterol molecules are exposed to polarized microscopy, the effect is such that a Maltese cross formation becomes visible, simplifying the identification process. Casts and red blood cells may be better visualized using phase-contrast microscopy. Ketone bodies will be soluble and, therefore, not seen in a urine sediment.

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**24.**

**D.** When neutrophils are exposed to hypotonic urine, their physical appearance becomes altered. Under hypotonic conditions, the neutrophils tend to swell and the cytoplasmic granules contained within the cells exhibit Brownian movement. This Brownian movement of the granules causes the neutrophilic contents to refract in such a way that the cells appear to glitter—thus the name “glitter cells.”

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**25.**

**D.** Waxy casts represent the final phase of granular cast degeneration. As the fine granules of the granular casts lyse, highly refractive, smooth, blunt-ended waxy casts are formed. When waxy casts are found in the urine sediment, the implication is that there is nephron obstruction caused by tubular inflammation and degeneration.

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**26.**

**C.** Nephrotic syndrome is suggested by the increased urine protein (with serum albumin significantly decreased), the hyaline and fatty casts, and the presence of oval fat bodies. The patient's symptoms of periorbital swelling and edema reflect the loss of oncotic pressure because of the excretion of albumin. Its loss from the vascular compartment will induce plasma water movement into the tissue spaces. Glomerulonephritis will have many more red blood cells, including red blood cell casts. Multiple myeloma will not show increased urine albumin but rather immunoglobulin light chains. Chronic renal failure will have multiple types of casts present (hyaline, granular, cellular, waxy, fatty).

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**27.**

**B.** There are minimal bacteria present in Color Plate 46■. Both budding yeast and white blood cells predominate this microscopic field. Patients with diabetes mellitus are prone to such yeast infections because of the increased glucose in their urine. The epithelial cells visualized in this field are transitional and not squamous. They can be distinguished by their size (about 15–20  $\mu\text{m}$ ), less cytoplasm than a squamous cell would have, and their central nucleus. Increased squamous epithelial cells would suggest improper collection, whereas transitional cells, if greater than 5 cells/hpf, would indicate pathology. There are fewer than 5 red blood cells in this field, and that would be below the sensitivity of the blood pad on the reagent strip.



28.

**D.** Alkaptonuria is a rare hereditary disease that is characterized by excessive urinary excretion of homogentisic acid. This acid, the product of phenylalanine and tyrosine metabolism, accumulates in urine because of a deficiency in the enzyme homogentisic acid oxidase, which normally catalyzes the oxidation of homogentisic acid to maleyl acetoacetic acid. Urine containing homogentisic acid turns black on standing because of an oxidative process; thus the screening test for alkaptonuria consists of the detection of a black coloration in urine that is left standing at room temperature for 24 hours.

29.

**C.** This student has a lower urinary tract infection (UTI), also known as cystitis. The major distinguishing features between upper and lower UTI include the presence of protein and casts in an upper UTI and not in a lower UTI. This is because both urine protein excretion and cast formation reflect what is happening within the kidney itself. The most common source of either upper or lower UTIs is contamination by enteric gram-negative bacteria. Their presence will not be found in glomerulonephritis or with urinary stones (nephro = “kidney” + lith = “stone”).

30.

**D.** The intestinal enterochromaffin cells, sometimes called the argentaffin cells, produce a substance known as serotonin from the amino acid tryptophan. In cases of metastatic carcinoid tumors, excessive amounts of serotonin are produced. Serotonin may then undergo oxidative deamination to form the metabolite 5-hydroxyindole acetic acid (5-HIAA), which is excreted in the urine. It is the quantification of 5-HIAA that is diagnostically significant because it reflects serotonin production.

31.

**B.** Because of increased lipid metabolism in long-term, intensive dieting, ketone body formation will increase. Blood glucose levels in such patients will be normal or decreased. In nephrotic syndrome, the large amounts of albumin excreted will be detectable by both reagent strip and SSA methods. In multiple myeloma, however, the increased globulin light chains (Bence Jones proteins) excreted will only be detectable by SSA because the reagent strip is more sensitive to albumin. Cystitis is a lower urinary tract infection affecting the bladder but not the kidney itself. This infection will not exhibit increased protein, whereas an upper urinary tract infection will.

32.

**C.** Bacteria of the *Enterobacter*, *Citrobacter*, *Escherichia*, *Proteus*, *Klebsiella*, and *Pseudomonas* species produce enzymes that catalyze the reduction of nitrate, a substance normally found in urine, to nitrite. Reagent test strips have been developed that are able to detect nitrite in urine. Therefore, a positive nitrite test result is an indirect indication of the presence of bacteria in the urine specimen.

33.

**C.** Cerebrospinal fluid (CSF) is a clear, colorless liquid that may be described as a modified ultrafiltrate of blood. Both active transport and passive diffusion are involved in the passage of glucose from the blood into the CSF. Normally, fasting CSF glucose levels range between 50 and 80 mg/dL, representing approximately 60–70% of the blood glucose level. In hyperglycemia with plasma glucose levels of 300 mg/dL, the active transport mechanism reaches a point of maximum response, so that CSF glucose levels reflect approximately 30% of the plasma glucose level. Decreased CSF glucose levels are associated with hypoglycemia, a faulty active transport mechanism, and excess utilization of glucose by microorganisms, red or white blood cells, or the central nervous system.

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**34.**

**C.** In addition to hemoglobin, the muscle protein myoglobin can cause a positive blood result in chemical reagent strip testing. Both hemoglobin and myoglobin possess pseudoperoxidase activity detected by the “blood” chemistry test. Muscle-crushing injuries (rhabdomyolysis) will release myoglobin from the muscle. The myoglobin, being a small molecule, is readily excreted by the kidneys. Myoglobinuria can lead to acute renal failure. Myoglobin can be distinguished from hemoglobin in urine by an ammonium sulfate screening test. Myoglobin will remain soluble in 80% ammonium sulfate and give a positive filtrate blood reaction after the precipitation of hemoglobin. The patient’s pathology involves neither red blood cells nor bilirubin. The severe stress may be responsible for both the urine protein and granular cast results.

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**35.**

**D.** The eGFR calculation is based on the “modification of diet and renal disease” formula recommended by the American Kidney Foundation. It does not use a urine sample at all, but instead requires only a serum creatinine and the patient’s age, gender, and race. Values less than 60 mL/min/1.73 m<sup>2</sup> are considered abnormal and need to be followed up. This patient’s value places him in stage 3 kidney damage (35–59 mL/min/1.73 m<sup>2</sup>). This calculation is considered more accurate than the Cockcroft-Gault formula, but there are limitations based on the standardization of the creatinine method used.

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**36.**

**A.** Normal alkaline (or neutral) urine may contain triple phosphate crystals, as seen in Color Plate 47■. These crystals can be identified by the characteristic “coffin lid” appearance. They usually do not indicate any pathology.

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**37.**

**D.** Refer to Color Plate 48■. The fringed appearance at the one end of the major formed element strongly suggests that this is a fiber artifact, most likely placed in the sample at the time of collection. Casts, taking the shape of the tubule within which they are formed, will not have such a fringed end.

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**38.**

**C.** The distal convoluted tubule and collecting duct provide water reabsorption through the action of antidiuretic hormone (vasopressin). The renin-angiotensin-aldosterone system is responsible for sodium reabsorption by the distal and collecting tubules. Decreased plasma volume leads to pressure alterations detected by receptors located in the kidney’s juxtaglomerular apparatus and the right atrium of the heart. These changes trigger the production of renin and antidiuretic hormone, respectively.

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**39.**

**A.** Only freshly voided urine specimens should be used for urinalysis testing. If the specimen cannot be examined within 1 hour after collection, it should be refrigerated to help preserve the integrity of the specimen. When urine is left standing at room temperature for an excessive period, multiplication of bacteria will occur. The bacteria are capable of converting urea in the urine to ammonia, causing the urine to become more alkaline. Loss of carbon dioxide from the specimen will also contribute to the alkalization of the urine. Constituents such as glucose, bilirubin, and urobilinogen will also be lost from the specimen.

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40.

**B.** The presence of cystine crystals in a patient sample is always a cause for immediate notification of the physician. Cystinuria is an autosomal recessive disorder characterized by the inability to reabsorb the amino acids cystine, lysine, arginine, and ornithine in either the renal tubules or the intestine. Cystine will crystallize in acid pH more readily than the other amino acids. Tyrosine forms needle-shaped crystals whereas leucine will appear round and oily with concentric rings. Isoleucine and phenylalanine will not form crystals in the urine. Acetoacetate and  $\beta$ -hydroxybutyric acid are two ketone bodies that will be soluble in the sample and give a positive reaction with nitroprusside.

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41.

**D.** The major formed elements in Color Plate 50■ are hyaline casts and mucus fibers, which are normal in the numbers shown in this field. Waxy casts will appear yellowish with characteristic serrated edges. There are no obvious granules in the casts shown, and red blood cells are not present.

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42.

**A.** Phenylketonuria is inherited as an autosomal recessive trait that manifests itself in the homozygous form. The basis for the disease lies in the fact that the enzyme phenylalanine hydroxylase, which is needed for the conversion of phenylalanine to tyrosine, is absent. Because of this enzyme deficiency, phenylalanine levels rise in the blood, with increased amounts of phenylpyruvic acid and other derivatives being excreted in the urine. If the disease is detected at an early stage, mental retardation may be avoided by restricting the dietary intake of phenylalanine.

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43.

**D.** Color Plate 51■ demonstrates sperm and calcium oxalate crystals. Both formed elements are found in correctly collected normal urines from either gender. Calcium oxalate seen here is the dehydrate form. The monohydrate form will appear oval or dumbbell shaped. Neither formed element is usually associated with pathology.

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44.

**B.** A variety of substances in CSF specimens have been associated with a xanthochromic appearance. Among those substances are oxyhemoglobin, carotenoids, bilirubin, and protein. The appearance of the specimen by itself is not usually specific for a particular disease state, but it may provide useful information in comparison with other findings. Glucose, magnesium, and chloride do not contribute to the color of the specimen.

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45.

**A.** Refer to Color Plate 52■. Erythrocytes or red blood cells (RBCs) occur in small numbers (0–2/hpf) in a normal urine. Using brightfield microscopy, unstained RBCs appear as colorless discs with an average size of 7  $\mu$ m in diameter. Increased or large numbers of RBCs are commonly seen with acute glomerulonephritis, renal calculi, acute infections, and menstrual contamination. The nephrotic syndrome is characterized by heavy proteinuria, oval fat bodies, renal tubular epithelial cells, casts, and waxy and fatty casts. Biliary tract obstruction will show pale-colored stools, whereas vaginal discharge contamination may introduce increased numbers of white blood cells.

46.

**A.** Because there may be technical problems associated with amniocentesis, contamination with maternal urine should be considered in evaluating specimens submitted for amniotic fluid analysis. Urinary concentrations of creatinine and urea nitrogen are anywhere from 10 to 50 times the amniotic fluid concentrations, and an increased concentration of either in the amniotic fluid would be sensitive indicators of urinary contamination. Measurements of albumin, total protein, or lactate dehydrogenase would be of little use for this purpose because their relative concentrations in urine and amniotic fluid are not predictably different. A delta absorbance at 410 nm would be used to assess the presence of bilirubin in the sample, as in assessment of erythroblastosis fetalis.

47.

**D.** The alveolar concentrations of the various phospholipids (surfactants) change during fetal lung development, and because these changes are reflected directly in the amniotic fluid, a number of investigations have shown that analysis of the fluid can provide good predictive information for the development of respiratory distress syndrome in the newborn. The concentrations of sphingomyelin and phosphatidyl inositol increase until about 32–34 weeks of gestation and then decline. Conversely, lecithin (phosphatidyl choline) and phosphatidyl glycerol concentrations increase rapidly after 32–34 weeks of gestation, and their concentrations relative to those of the other phospholipids are useful in assessing the development of fetal lung maturity.

48.

**C.** The hemoglobin released from red blood cells in an upper gastrointestinal bleed will have time to become denatured and oxidized as it travels the entire intestinal tract. This will make the stool become much darker in color by the time the hemoglobin is excreted. Clay-colored

stools will result from an obstruction of the biliary duct, preventing bilirubin from entering the intestines to be converted into urobilinogen and then into urobilin. Stools with red streaks are more likely to result from a lower gastrointestinal bleed, as from the colon. Increased mucus will be associated with intestinal inflammation.

49.

**B.** Effusions can be transudates or exudates, and the distinguishing characteristics are cell number and total protein. Transudates, being noninflammatory, will have low numbers of cells and less than 3 g/dL protein. This patient's results suggest she has an exudate due to the high number of cells and large amount of protein.

50.

**A.** Diabetes insipidus is caused by a deficiency in antidiuretic hormone. Such deficiencies will result in the kidney's inability to reabsorb water at the distal and collecting tubules. This affects only water reabsorption and not the reabsorption of other urinary solutes. Excreted solute amounts will be the same, but the water volume into which they are excreted will be larger. This results in high urine volumes and low final solute concentrations. The low solute will lead to low specific gravities in these patients' specimens.

51.

**D.** Synovial fluid is a form of plasma ultrafiltrate with added hyaluronic acid. Decreased viscosity and poor mucin clot formation are indications of the decreased hyaluronate concentration of synovial fluid. Either of these findings is usually an indication of inflammation. Because the viscosity of synovial fluid is normally very high, it can be estimated by the length of string formed when the fluid drops from a syringe. The term "mucin" in the mucin clot test is a misnomer, because mucin is not present in synovial fluid.



52.

**A.** Effusions result from an imbalance of the flow of body fluids. Effusions are classified as exudates or transudates on the basis of certain characteristics. Exudates are generally formed in response to inflammation or infection with concomitant capillary wall damage. Exudates are characterized by protein levels greater than 3.0 g/dL, leukocyte counts greater than 1000/ $\mu$ L, and the presence of a sufficient amount of fibrinogen to cause clotting. In contrast, transudates are characterized by protein levels less than 3.0 g/dL, leukocyte counts less than 300/ $\mu$ L, and the absence of fibrinogen. Transudates are generally formed as the result of noninflammatory processes, including alterations in plasma oncotic pressure, pleural capillary hydrostatic pressure, or intrapleural pressure.

53.

**D.** A clinically useful test for assessing the concentrating and diluting ability of the kidneys is the determination of urine specific gravity. The specific gravity is a measure of the proportion of dissolved solids in a given volume of solvent. Polyelectrolytes are incorporated into urinalysis reagent strips. A dye also present in the strips will change color because of a pKa change in the polyelectrolytes. The pKa varies with the ionic concentration of the urine. The color obtained is compared with a set of standard colors, each color correlating with a different specific gravity concentration. The TS meter is a specific type of refractometer that utilizes the close correlation of a solution's refractive index with its solute concentration to determine the specific gravity of urine. The refractive index is the ratio of the velocity of light in air to the velocity of light in a solution, this being comparable to the number of dissolved particles in that solution. An osmometer measures the concentration of dissolved solute in the sample, usually through its relationship to freezing point depression.

54.

**D.** Trichloroacetic acid is a turbidimetric method used to quantify small amounts of protein, less than 100 mg/dL, in cerebrospinal fluid (CSF) and urine specimens. Coomassie brilliant blue is a colorimetric dye binding method in which protein complexes with the dye, forming a soluble blue complex. This method also exhibits the necessary sensitivity for detecting small quantities of protein. Bromocresol green is selective for albumin and is used to quantify albumin in serum. Ponceau S is used in serum protein electrophoresis methods to stain both albumin and globulins.

55.

**A.** As seen in Color Plate 53■, hyaline casts are the most commonly observed cast, and they consist completely of uromodulin (Tamm-Horsfall) protein. A reference urine may contain 0–2 hyaline casts per low-power field. Hyaline casts appear translucent using brightfield microscopy because they have a refractive index similar to urine. Phase-contrast microscopy may be used to visualize the casts better.

56.

**A.** To be found in urine, a solute must be water soluble. Solutes can be inorganic (e.g., sodium) or organic (e.g., urea). Excreted waste products, meaning end products of metabolism, are creatinine, urea, and uric acid. Some excreted solutes, however, are not present as waste but as overload, such as glucose or sodium.

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**57.**

**D.** Cerebrospinal fluid (CSF) must be collected in sterile tubes. The first tube is generally used for chemistry and serology studies, the second tube is employed for bacteriologic examination, and the third tube is used for cell counts. Tubes used for chemistry and bacteriologic studies should be centrifuged before use. CSF should remain uncentrifuged for cell counts. Low-volume specimens need to be cultured first (to ensure sterility) before any other test is performed. Because the analysis of CSF should be performed immediately, it is critical that personnel on all shifts be able to perform the necessary testing.

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**58.**

**B.** Uric acid crystals, as seen in Color Plate 54■, are commonly encountered in normal acidic urine but may be observed in neutral urine and rarely in an alkaline urine, because uric acid is soluble at alkaline pH. Using brightfield microscopy, uric acid crystals appear as diamonds, cubes, barrels, rosettes, and may even have six sides and be confused with cysteine. Because they are a reflection of the excretion of purine waste products, they may be pathologically increased in cases of gout and after chemotherapy. They show birefringence (multiple colors) under plane polarized light.

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**59.**

**A.** A positive urine glucose plus a positive serum ketone strongly suggest uncontrolled diabetes mellitus. There is an increased rate of fatty acid oxidation occurring in light of the inaccessibility of the glucose, especially to skeletal muscle. If the patient had only been dieting, the glucose would be negative.

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**60.**

**B.** The sensitivity of a method is the lowest concentration of the analyte that will result in a detectable reaction signal. The protein, bilirubin, and nitrite readout color scales each have a color

associated with analyte concentrations less than the method's sensitivity, called "negative." Urobilinogen's readout color scale begins with its lowest reportable value, but there is no pad associated with concentrations less than this.

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**61.**

**A.** In the hepatic phase of bilirubin metabolism, bilirubin is conjugated with glucuronic acid to form water-soluble conjugated bilirubin. The conjugated bilirubin passes into the bile duct and on to the intestinal tract. In the intestine, it is reduced by intestinal bacteria to form urobilinogen. Bile duct obstruction is characterized by an obstruction of the flow of conjugated bilirubin into the intestinal tract to complete its metabolism. The conjugated bilirubin, which is water soluble, will be excreted by the kidney. Because bilirubin is not entering the intestines, the normal production of urobilinogen is decreased. Therefore, the urine biochemical test will indicate a positive reagent strip test for bilirubin, positive Ictotest, and "normal" (0.2 mg/dL) urobilinogen (because there is no reagent strip pad for "negative" urobilinogen).

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**62.**

**C.** DNA-based tests for detecting mutations within colon cells are generally more expensive than the fecal occult blood (FOB) methods using the pseudoperoxidase property of hemoglobin. Advantages, however, include no diet or medication restrictions prior to the testing, use of a single stool sample, and a sensitivity (50–73%) that far exceeds that of the FOB test (13–35%) for detecting colon cancer. It is, however, less sensitive than colonoscopy, which can detect 95% of colon cancers. Gene mutations associated with colorectal cancer include APC (adenomatous polyposis coli) on chromosome 5; K-ras, a mutation that often occurs after APC mutation; and p53, with mutations occurring later in the process, associated with larger adenomas.

63.

**C.** The morphologic characteristics of spermatozoa are best evaluated by means of smears stained with Papanicolaou stain. Other stains used include Kernechtrot, Giemsa, basic fuchsin, crystal violet, and hematoxylin. When oil immersion is used, a minimum of 200 spermatozoa should be evaluated for morphologic characteristics. Although sources differ as to the exact number, it is generally established that at least 60% of the sperm should have normal morphologic features. When this microscopic analysis is performed, the presence of erythrocytes, leukocytes, epithelial cells, and microorganisms should be indicated.

64.

**D.** Immunoglobulins (IgGs) are normally present at less than 1 mg/dL in the CSF. Increased CSF IgG can result from increased CSF production (e.g., multiple sclerosis) or from increased transport from the blood plasma (compromised blood-brain barrier). Neither gout, erythroblastosis fetalis (isoimmunization syndrome), nor multiple myeloma produces increased CSF IgG levels.

65.

**A.** Many simplified yet immunologically sophisticated methods exist currently for determining pregnancy. All are based on the reaction between patient human chorionic gonadotropin (hCG) and anti-hCG. Most kits will use an antibody recognizing one subunit of hCG (alpha or beta), whereas other kits may use both anti- $\alpha$ -hCG and anti- $\beta$ -hCG. Internal controls in these kits will only check if the procedural steps were performed correctly. They cannot detect problems with any preanalytical variables, like specimen handling or appropriateness. In addition, internal quality control cannot be used to assess the kit's accuracy in

distinguishing "positive" from "negative" specimens. Only the use of external quality control specimens can accomplish this. Because the first morning specimen is the most concentrated of the day, it is the preferred specimen for such screenings. Use of a random urine may be too dilute to detect low levels of patient hCG, thus giving a false negative.

66.

**D.** Galactosemia, an inborn error of metabolism, is characterized by the inability to metabolize galactose, a monosaccharide that is contained in milk as a constituent of the disaccharide lactose. Thus galactose appears in elevated levels in the blood and urine. The condition may result in liver disease, mental retardation, and cataract formation if not treated or controlled. In the biochemical analysis of the urine, the conflicting results for the two glucose tests may be explained as follows: The glucose oxidase reagent strip test is specific for glucose; therefore, the glucose will be negative. The Clinitest<sup>®</sup>, a modification of the Benedict's test procedure, detects most reducing substances. Because galactose is present in the urine and is a reducing substance, the Clinitest<sup>®</sup> is positive.

67.

**C.** Renal tubular cells originate from the renal medulla or cortex. Red blood cell crenation is a phenomenon reflecting increased solute concentration (hyperosmolality) and is not caused by urine pH. Red cells will, however, lyse at high alkaline pH. The nitrite reaction requires (a) a sufficient dietary source of nitrate, (b) sufficient numbers of bacteria present in the urine, and (c) sufficient incubation time (>4 hours). Bacteria introduced at collection, even in sufficient number, will not have had sufficient incubation time to convert urine nitrate to nitrite. Pilocarpine iontophoresis is the collection method for sweat.



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